6 Plant Phenolics

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1 Introduction

The expression "phenolic compounds" embraces a considerable range of substances which possess an aromatic ring bearing a hydroxyl substituent, and while a significant number of such compounds occur in animals, most are of plant origin. Indeed, the presence of a "phenolic fraction" is a characteristic feature of all plant tissues. Among the plant polyphenols of which several thousand have now been described, the flavonoids form the largest group. However, phenolic quinones, lignans, xanthones and coumarins and other groups exist in considerable numbers and there

Table 1. The major classes of phenolics in plants

Number of carbon atoms	Basic skeleton	Class	Examples
6	C_6	Simple phenols Benzoquinones	Catechol, hydroquinone 2,6-Dimethoxybenzoquinone
7	C_6-C_1	Phenolic acids	*p*-Hydroxybenzoic, salicylic
8	C_6-C_2	Acetophenones Phenylacetic acids	3-Acetyl-6-methoxybenzaldehyde *p*-Hydroxyphenylacetic
9	C_6-C_3	Hydroxycinnamic acids Phenylpropenes Coumarins Isocoumarins Chromones	Caffeic, ferulic Myristicin, eugenol Umbelliferone, aesculetin Bergenin Eugenin
10	C_6-C_4	Naphthoquinones	Juglone, plumbagin
13	C_6-C_1-C_6	Xanthones	Mangiferin
14	C_6-C_2-C_6	Stilbenes Anthraquinones	Lunularic acid Emodin
15	C_6-C_3-C_6	Flavonoids Isoflavonoids	Quercetin, cyanidin Genistein
18	$(C_6$-$C_3)_2$	Lignans Neolignans	Pinoresinol Eusiderin
30	$(C_6$-C_3-$C_6)_2$	Biflavonoids	Amentoflavone
n	$(C_6$-$C_3)_n$ $(C_6)_n$ $(C_6$-C_3-$C_6)_n$	Lignins Catechol melanins Flavolans (Condensed tannins)	– – –

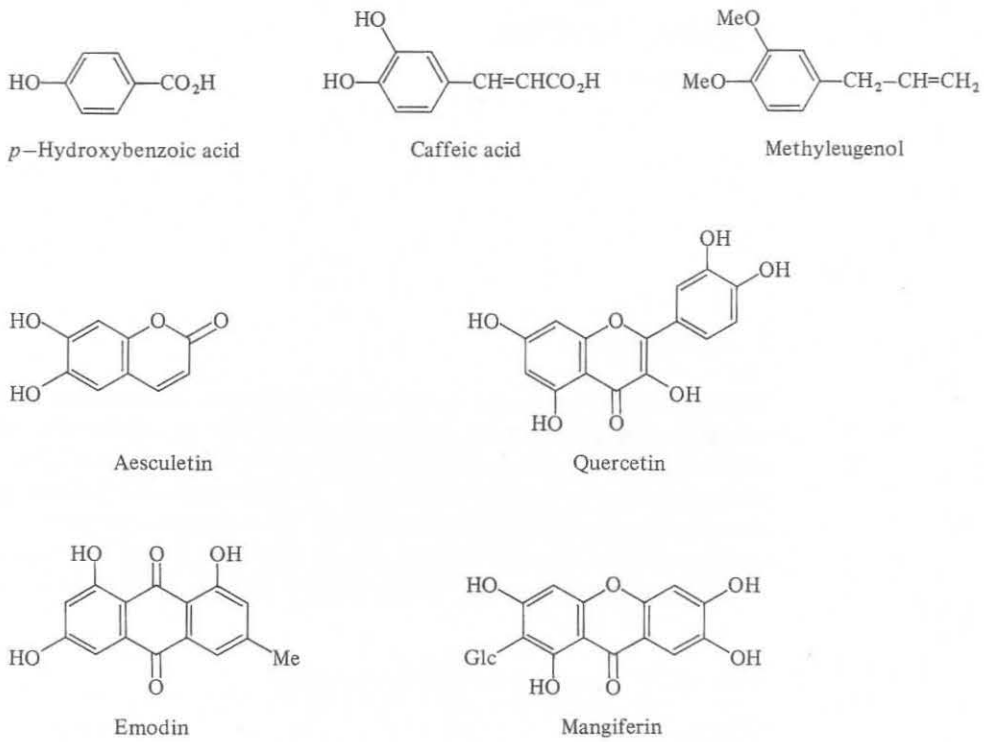


Fig. 1. Structures of some commonly occurring plant phenolics

are also many simple monocyclic phenols. In addition to monomeric and dimeric structures, there are also three important groups of phenolic polymer – the lignins, melanins, and tannins. Phenolic units are also encountered among nitrogen compounds (for example the aromatic amino acid tyrosine is phenolic) and among terpenoids.

Most of the major classes of plant polyphenol are listed in Table 1, according to the number of carbon atoms of the basic skeleton. Under the term "phenolic" are included compounds where this function may be masked by *O*-methylation or other substitution. Many of the compounds listed contain other functional groups besides phenolic hydroxyl groups and this clearly modifies their properties on occasion. Some illustrative structures of the more commonly occurring plant polyphenols are shown in Figure 1.

The seemingly bewildering array of natural phenols forms a more coherent picture when biogenetic considerations are taken into account. Practically all higher plant polyphenols are formed from shikimate, via the shikimic acid pathway and their production through the intermediacy of phenylalanine, the enzyme phenylalanine ammonia lyase, and cinnamic acid is illustrated in Figure 2. The role of acetate-malonate units in biosynthesis should not, however, be ignored, since these are needed, together with phenylpropanoid moieties, for the production of flavonoids. Many simple phenols and many plant quinones are also derived directly by the polyketide pathway from acetyl and malonyl coenzyme A.

Chemically, phenols are reactive substances and this must be borne in mind when considering their functions in plants. They are usually acidic and can often be separated from other plant constituents by their solubility in aqueous sodium carbonate. Unless sterically hindered, all phenols are capable of taking part in hydrogen bonding. This may be intramolecular, as between the 5-hydroxyl and 4-carbonyl in many flavonoids. More importantly, it may be intermolecular and bring about interactions between plant phenols and the peptide links of proteins and enzymes. Another important property of the many phenols with an *o*-dihydroxy (catechol) grouping is their ability to chelate metals and such metal chelates may be important biologically in plant systems. Finally, phenols are very susceptible to oxidation and there are special enzymes present in plants – the phenolases – which catalyse the oxidation of monophenols to diphenols and of diphenols to quinones and hence often to polymeric materials.

One of the problems in establishing a physiological role for plant polyphenols is the multitude of structures encountered in nature. It should, however, be emphasised that only a small number of compounds (cyanidin, quercetin, caffeic acid, etc.) are at all widespread; most of the others are of very limited occurrence. In terms of growth regulation, phenolics seem commonly to be inhibitors. Thus, the only established hormone with phenolic substitution – lunularic acid – is an inhibitor of growth and occurs throughout the liverworts, where it apparently replaces abscisic acid, the more universal dormancy hormone. The interaction between plant hormones and other classes of phenol has been under increasing study and while most of these experiments have been carried out in vitro, there are those who believe that phenols of higher plants may have an important controlling effect on plant growth in vivo.

In Volume X of the first *Encyclopedia of Plant Physiology*, there was very generous coverage of phenolics and chapters were included on anthocyanins, tannins, lignins, lichen phenols, and on biogenesis and metabolism. Since that time, progress in the study of plant phenols has been enormous and it is impossible in the space provided to cover the advances of the last twenty years at all comprehensively. Only a few topics can be selected for detailed treatment here. After a survey of the structural variation among the different classes of polyphenol, consideration will be given to the different ways that these compounds are conjugated in plant cells. A summary will then be given of their pathways of biosynthesis, their enzymology, and further metabolism. Their production in tissue culture will be compared with their formation in intact plants. Finally, an attempt will be made to summarise what is known of their functions in plants.

2 Phenolic Aglycones

2.1 Simple Phenols and Phenolic Acids

Although most of the more complex and widely occurring plant polyphenols contain catechol (I) or phloroglucinol (II) units as parts of their structures (see Fig. 1), these two simple phenols are relatively uncommon and of scattered occurrence. Catechol, in fact, has been reported with certainty in only a few plants, e.g.,

in leaves of three *Gaultheria* species as the glucoside (TOWERS et al., 1966). It may, however, be more frequently produced than this, since there is evidence (NICOLAUS and PIATELLI, 1965) that most plant melanins are catechol-based and are formed by oxidative polymerisation of catechol in the presence of the enzyme phenolase. Such black pigments, which yield catechol on degradation, have been found for example in seeds of sunflower and water melon and in the rust spores of the fungus *Ustilago maydis*. Finally, catechol derivatives with aliphatic substituents are known; urushiol (III), with a C_{15} hydrocarbon side chain, is the vesicant principle of poison ivy *Rhus toxicodendron* (Anacardiaceae).

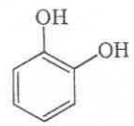
Phloroglucinol (II) is also quite rare in plants, although it does occur as its glucoside, phlorin, in the peel of various *Citrus* fruits. Derivatives of phloroglucinol are also uncommon. Acylphloroglucinols occur in rhizomes of a few *Dryopteris* species (Filicopsida) and the hop resin compounds humulone and lupulone in *Humulus lupulus* are isoprenoid substituted phloroglucinols (SWAIN and COOPER-DRIVER, 1973).

Of other simple phenols in nature, the most widespread is undoubtedly hydroquinone (IV), which occurs in several families but is especially associated with *Pyrus* and *Docynia* in Rosaceae and with the tribe Arbuteae of Ericaceae (HARBORNE and WILLIAMS, 1973). Resorcinol itself does not appear to have been found in plants, but its 5-methyl derivative, orcinol (V) is present in *Erica arborea* and in a number of other Ericaceae (HARBORNE and WILLIAMS, 1969). A more complex natural derivative of resorcinol is Δ^1-tetrahydrocannabinol (VI) which is the hallucinogenic principle of leaves and flowers of *Cannabis sativa* (MECHOULAM, 1970).

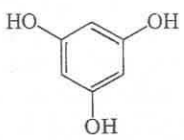
By contrast with the simple phenols, phenolic acids seem to be universally distributed in plants; at least, acid or alkaline treatment of plant extracts normally yields a mixture of several such acids. The mode of binding is not entirely clear, but the acids are mainly present in the "alcohol-insoluble" fraction of leaf tissue and are probably at least partly linked to lignin by ester bonds. Four acids are apparently universal in the angiosperms: *p*-hydroxybenzoic (VII), protocatechuic (VIII), vanillic (IX) and syringic acids (X). The first three are also present widely in gymnosperms and ferns. The presence of bound syringic acid in leaves is correlated with the occurrence of syringyl residues specifically in angiosperm lignins (IBRAHIM et al., 1962).

Two other common acids in angiosperms are gentisic (XI) and salicylic acid (XII). Salicylic acid is occasionally present as its methyl ester in essential oils and this ester is the pungent-smelling oil of wintergreen of the British Pharmacopoeia. Salicylic acid is especially widespread in Ericaceae, where it occurs in association with *o*-pyrocatechuic acid (2,3-dihydroxybenzoic acid).

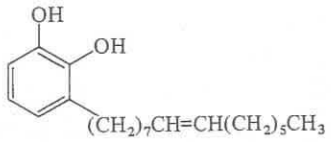
Gallic acid (XIII) is another well-known plant acid. It has recently been characterised as a specific flowering-inhibitor in leaves of *Kalanchoe blossfeldiana* and is also present in a bound form in the same plant after flowering (PRYCE 1972c). In its more usual occurrence in woody plants, gallic acid occurs in soluble form linked to glucose, as hydrolysable gallotannin. Its dimeric condensation product, hexahydroxydiphenic acid and the related dilactone, ellagic acid (XIV) are also common plant constituents, the former usually occurring bound as ellagitannin. Hydrolysable tannins are regularly present in woody dicotyledonous (but not monocotyledonous) angiosperms, occurring especially in a cluster of families with Rosalian affinities (BATE-SMITH, 1972).



Catechol
(I)

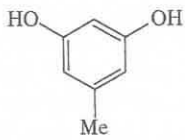


Phloroglucinol
(II)

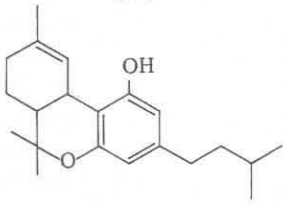


Urushiol
(III)

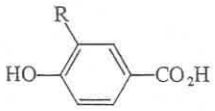
Hydroquinone
(IV)



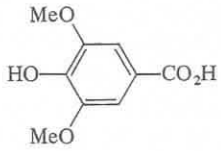
Orcinol
(V)



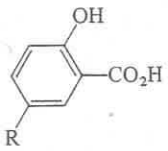
Δ^1–Tetrahydrocannabinol
(VI)



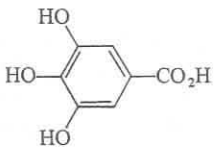
R = H, *p*–Hydroxybenzoic acid (VII)
R = OH, Protocatechuic acid (VIII)
R = OMe, Vanillic acid (IX)



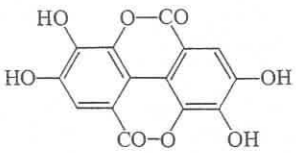
Syringic acid
(X)



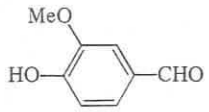
R = OH, Gentisic acid (XI)
R = H, Salicylic acid (XII)



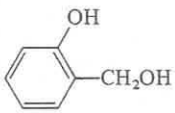
Gallic acid
(XIII)



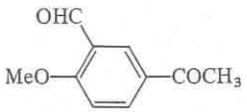
Ellagic acid
(XIV)



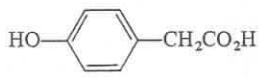
Vanillin
(XV)



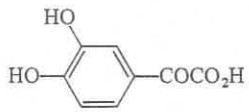
Salicylalcohol
(XVI)



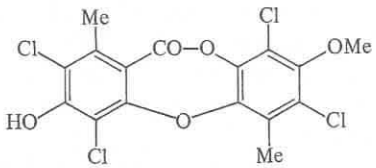
(XVII)



p–Hydroxyphenylacetic acid
(XVIII)



3,4–Dihydroxyphenyl-
glyoxylic acid
(XIX)



Diploicin
(XX)

Aldehydes related to the common C_6-C_1 acids are found in plants; for example, vanillin (XV) is the odour principle of vanilla pod *Vanilla planifolia* (Orchidaceae) and is present in a variety of plants. The corresponding alcohols are also known, an example being salicylalcohol (XVI) present in willow, *Salix* and in certain Rosaceae. Related phenolic ketones are also occasional plant products. One of particular ecological interest is 3-acetyl-6-methoxybenzaldehyde (XVII) which occurs as a growth inhibitor in leaves of the desert shrub *Encelia farinosa* and persists in the soil surrounding the plant causing the inhibition of germination in the seeds of annuals growing in the vicinity (GRAY and BONNER, 1948).

Related to the acetophenones, in being C_6-C_2 compounds, are the phenylacetic acids. Both the 2- and 4-hydroxyderivatives have been identified in plants, in *Astilbe* leaves and in roots of dandelion (*Taraxacum officinale*) respectively. The 4-hydroxy compound (XVIII) has recently been claimed as a natural growth regulator in certain higher plants (see GROSS, 1975) and also as an auxin-like substance in an alga (ABE et al., 1974).

A wide range of simple phenols and phenolic derivatives has also been found in fungi and lichens; fungal phenols have been listed by TURNER (1971) and lichen compounds by CULBERSON (1969). Two phenols which are unique to fungi are 3,4-dihydroxyphenylglyoxylic acid (XIX) of *Polyporus tumulosus* and 3,4,5-trihydroxybenzaldehyde of *Boletus scaber*. Illustrative of the more complex phenols of lichens is diploicin (XX), a depsidone from *Diploicia canescens*, which is biogenetically derived by esterification of a substituted 2,4-dihydroxybenzoic acid with a resorcinol derivative. Its structure, as with other lichen constituents, is complicated by nuclear substitution in the aromatic rings by methyl and chlorine groups.

2.2 Phenylpropanoids

As is apparent from Figure 2, a ubiquitous phenolic unit in plants is one with an aromatic ring attached to a C_3 aliphatic side-chain. Such phenylpropanoids may be exemplified by *p*-hydroxycinnamic acid (or *p*-coumaric acid) (XXI) which occurs universally in plants and is also the precursor of many other similar C_6-C_3 molecules. By *o*-hydroxylation and subsequent cyclisation, *p*-coumaric acid can give rise to hydroxycoumarins. On reduction, it yields *p*-coumaryl alcohol, which is one of the monomeric "building blocks" of the secondary cell wall polymer lignin. Dehydrogenation of this alcohol gives a phenylpropene, present as a group in volatile oils of plants. Dimerisation of *p*-coumaryl alcohol can give rise to a lignan. Finally, the *p*-coumaric acid moiety is an important unit in the structure of flavonoids, stilbenes and xanthones (Fig. 2). All the above classes of phenylpropanoid occur widely in plants.

The most common hydroxycinnamic acid of plants is caffeic acid (XXII) which is universal in higher plants; it is usually present as the depside chlorogenic acid, but many other combined forms are known. Besides *p*-coumaric and caffeic, two methylated acids are also very common: ferulic (XXIII) and sinapic (XXIV). Ferulic acid, in particular, can be found in many different contexts. It has been identified as an *N*-acyl terminal group in a protein in barley seed (VAN SUMERE et al., 1973), linked directly to glycine and phenylalanine. It also occurs, dimerised as diferulic acid (XXV), bound to the carbohydrate of cell walls in a number of grasses

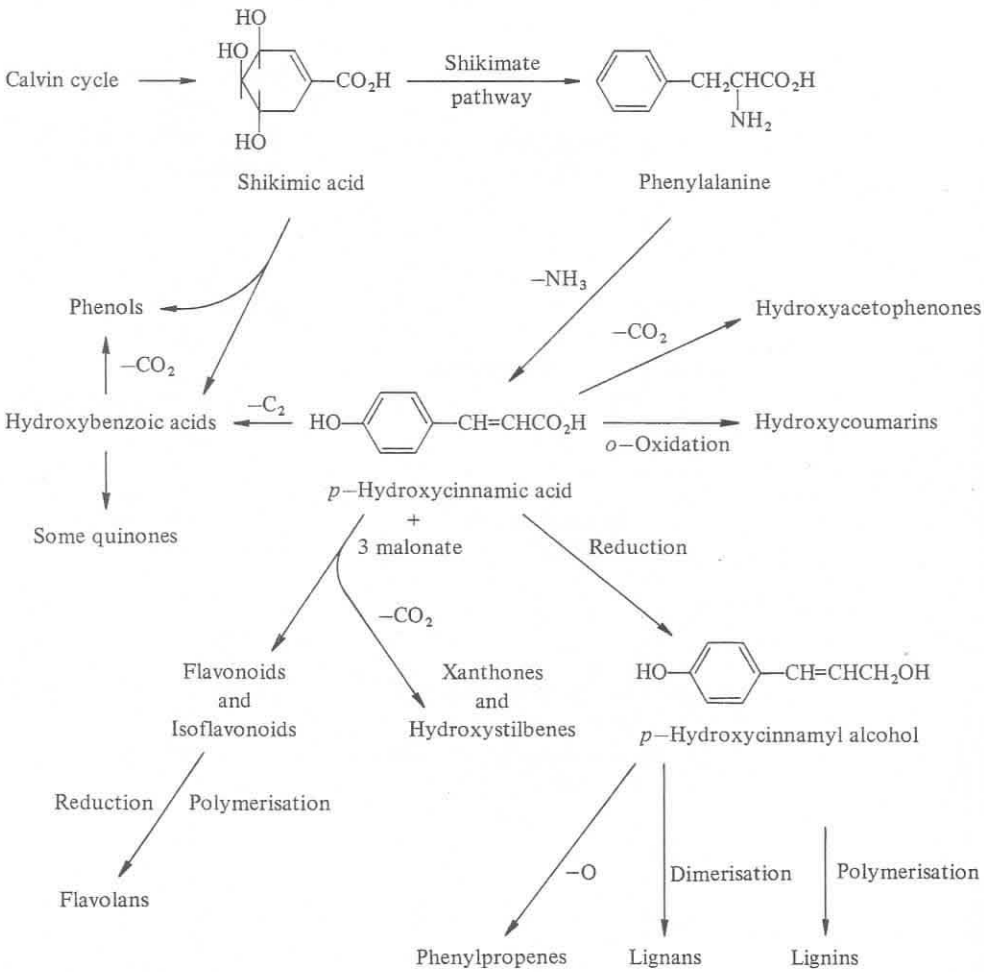
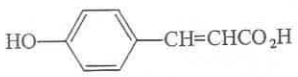


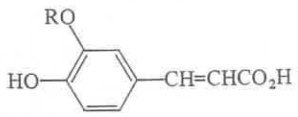
Fig. 2. Biosynthetic origin of plant phenolics from shikimate and phenylalanine

(HARTLEY and JONES, 1976). Again, ferulic acid is present free in sugar beet and cereal seeds and is reputedly a general germination inhibitor (VAN SUMERE et al., 1972). Hydroxycinnamic acids and their derivatives are capable of existing in cis and trans forms, and while there is evidence that the natural forms are all trans, isomerisation inevitably occurs during extraction, and mixtures of isomers are always isolated. Artefactual oxidation in the *o*-position can also occur and caffeic acid, in particular, may be partly converted to the coumarin, aesculetin (see below), during the isolation process.

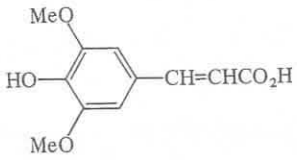
Phenylpropionic acids, formed by reduction of the aliphatic double bonds of cinnamic acids, have been detected in nature. Melilotic acid (dihydro-*o*-coumaric acid) occurs in the Leguminosae, and dihydrocaffeic acid in beetroot (Chenopodiaceae). The related 3,4-dihydroxyphenyl-lactic acid (XXVI) occurs naturally as its caffeic acid ester (rosmarinic acid) which was first isolated from rosemary *Rosmarinus officinalis* (Labiatae) but is now known to be widespread in this and related families (HARBORNE, 1966).



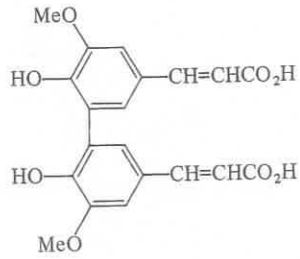
p–Coumaric acid
(XXI)



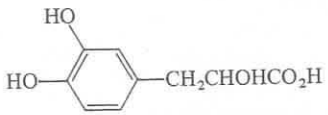
R = H, caffeic acid (XXII)
R = Me, ferulic acid (XXIII)



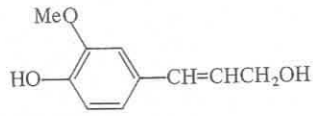
Sinapic acid
(XXIV)



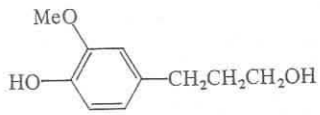
Diferulic acid
(XXV)



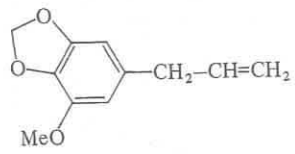
3,4–Dihydroxyphenyl–lactic acid
(XXVI)



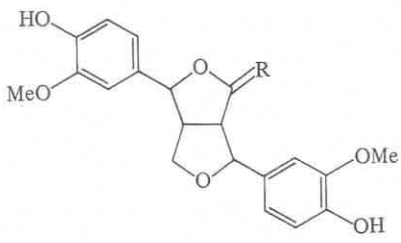
Coniferyl alcohol
(XXVII)



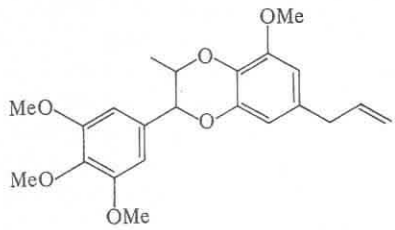
Dihydroconiferyl alcohol
(XXVIII)



Myristicin
(XXIX)



R = O, *Aegilops* lignan (XXXI)
R = H_2, pinoresinol (XXX)



Eusiderin
(XXXII)

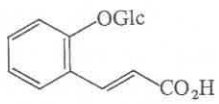
The three alcohols corresponding to *p*-coumaric, ferulic, and sinapic acids are undoubtedly widespread in plants, at least as trace constituents, since they are known to be the immediate precursors of plant lignins (GROSS, 1978). In keeping with this role, coniferyl alcohol (XXVII), the ferulic acid analogue, has been found in cambial sap of both angiosperms and gymnosperms, while sinapyl alcohol occurs similarly only in angiosperm tissues. Dihydroconiferyl alcohol (XXVIII) has recently been reported as a plant product, since it has been identified as the "lettuce cotyledon factor" in *Lactuca*. It is a synergist of gibberellic acid in inducing elongation of hypocotyls and has this activity in lettuce and in several other plants (SIKURAI et al., 1974).

The common plant hydroxycinnamyl alcohols are also precursors of two further classes of monomeric phenylpropanoid: allyl and propenylphenols. Thus coniferyl alcohol (XXVII) has been shown to be the immediate precursor of eugenol (side-chain-$CH_2-CH=CH_2$) and its methyl ether, methyleugenol, which occur together in leaves of the herb basil, *Ocimum basilicum* (KLISCHIES et al., 1975). Allyl and propenyl phenols occur sporadically in higher plants, in essential oil fractions of leaves and fruits, but are not necessarily found together as isomeric pairs. One of the most widely occurring is myristicin (XXIX) which is reputedly hallucinogenic in man (SHULGIN, 1966). While present in nutmeg *Myristica fragrans* (myristicaceae) together with isomyristicin, it occurs on its own in a range of umbellifers (HARBORNE et al., 1969).

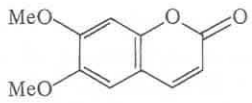
Dimers, formed from the union of two coniferyl or sinapyl alcohol units, are known as lignans. Pinoresinol (XXX), for example, is derived by tail-to-tail linkage in the β-position of two coniferyl alcohol residues and occurs widely in *Pinus* and *Picea* (HATHWAY, 1962). The ketone (XXXI) derived from (XXX) has recently been characterised as one of the germination inhibitors in seeds of the cereal *Aegilops ovata* (COOPER et al., 1977). Related dimers, called neolignans, can be formed by other condensations between two C_6-C_3 units, e.g., joining head-to-tail instead of tail-to-tail. One such compound is eusiderin (XXXII) and this and related structures occur in heartwoods of Magnoliaceae, Piperaceae, and Lauraceae (GOTTLIEB, 1972).

Coumarins are lactones formally derived from *o*-hydroxycinnamic acids by cyclisation and ring closure between the *o*-hydroxy and carboxyl groups. Coumarin itself occurs in fresh plant tissues (e.g., in leaves of *Melilotus alba*) in bound form as the corresponding trans-*o*-glucosyloxycinnamic acid (XXXIII). On tissue damage, this bound form undergoes enzymic loss of sugar, trans → cis isomerisation and ring closure, the volatile coumarin being released from the leaf surface as "the odour of new mown hay". Some methoxycoumarins occur naturally, bound in the same way. Thus 6,7-dimethoxycoumarin (XXXIV) is present in leaves of the orchid *Dendrobium densiflorum* as the corresponding 2-glucosyloxy-4,5-dimethoxycinnamic acid (DAHMEN et al., 1975). By contrast, the common hydroxycoumarins occur in plants as such, with *O*-glycosidic attachment. Indeed, such glycosides (e.g., aesculin) have an intense blue fluorescence and their presence in plant tissues (e.g., bark of horse chestnut) can be readily detected by examining the material in UV light.

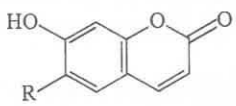
The three most common hydroxycoumarins of plants are umbelliferone (XXXV), aesculetin (XXXVI), and scopoletin (XXXVII) and correspond in structure to *p*-coumaric, caffeic, and ferulic acids. Curiously, the coumarin corresponding



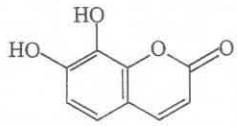
(XXXIII)



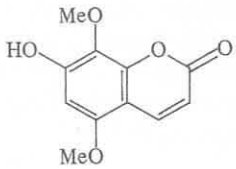
6,7–Dimethoxycoumarin
(XXXIV)



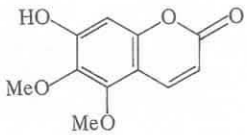
R = H, Umbelliferone (XXXV)
R = OH, Aesculetin (XXXVI)
R = OMe, Scopoletin (XXXVII)



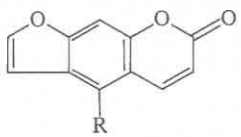
Daphnetin
(XXXVIII)



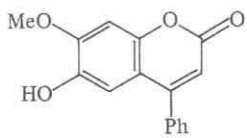
Leptodactylone
(XXXIX)



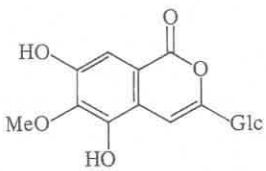
(XL)



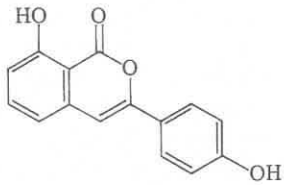
R = OMe, Bergapten (XLI)
R = H, Psoralen (XLII)



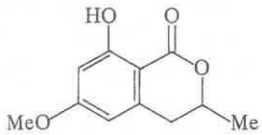
Dalbergin
(XLIII)



Bergenin
(XLIV)



Hydrangenol
(XLV)



Carrot isocoumarin
(XLVI)

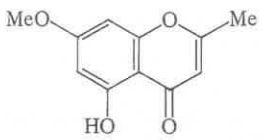
to sinapic acid, isofraxidin (6,8-dimethoxy-7-hydroxycoumarin), is quite rare, being restricted to bark of *Fraxinus* (Oleaceae). A fairly common coumarin, which has no obvious cinnamic acid counterpart, is daphnetin (XXXVIII), which was first isolated as the glucoside, daphnin, from bark of *Daphne mezereum* (Thymelaceae). It is also known from several other families and has recently been detected for the first time in Polemoniaceae (*Linanthus* and *Leptodactylon*) and in Juncaceae (*Juncus effusus*) (WILLIAMS and HARBORNE, 1975). Among other recently reported coumarins are leptodactylone (XXXIX), a yellow pigment in leaves of *Leptodactylon* (DEAN et al., 1978) and 5,6-dimethoxy-7-hydroxycoumarin (XL), present in roots of *Pelargonium reniforme* (Geraniaceae) (WAGNER and BLADT, 1975).

While structural variation among hydroxycoumarins is limited, there are more complex plant coumarins, known as furocoumarins, of which an almost infinite variety of structures has been described. These are mainly derived from umbelliferone (XXXV) by condensation with C_5 isoprenoid units and they are usually lipid-soluble, most or all phenolic hydroxyls being protected by *O*-methylation or isoprenylation. Bergapten (XLI) from bergamot oil is but one of several hundred such substances. Furocoumarins are of physiological interest because of their growth-inhibiting effects in relation to seed germination. Thus, psoralen (XLII) has been identified as an endogenous inhibitor in the seedcoat of *Psoralea subacaulis* (Leguminosae). It inhibits germination of the seed and has to be leached out of the coat before the seed will develop. On leaching into the soil, it may also prevent germination of the seed of other plant species in the vicinity (BASKIN et al., 1967). A chemical account of the furocoumarins is provided by DEAN (1963). More recent reviews on the structural variation and natural distribution of these compounds in Umbelliferae and Rutaceae can be found in HEYWOOD (1971) and GRAY and WATERMAN (1978).

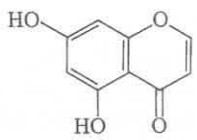
One final class of phenolic coumarin should be mentioned: 4-phenylcoumarins or neoflavanoids. A typical member is dalbergin (XLIII) which occurs in heartwood of *Dalbergia* trees (Leguminosae). About 42 structures are known and they are found almost exclusively in plants of the Leguminosae or Guttiferae (DONNELLY, 1975).

Only a few isocoumarins are known, but all carry one or more phenolic groups. One is bergenin (XLIV), in roots of *Astilbe, Bergenia, Peltoboykinia*, and *Rodgersia* (Saxifragaceae). Another is hydrangenol (XLV), a 3-phenyl substituted isocoumarin present specifically in *Hydrangea macrophylla* (BILLEK and KINDL, 1962). A third, 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (XLVI), is of plant pathological interest, because although a fungal metabolite, it is formed in carrot, *Daucus carota*, tissue following infection by the pathogen *Ceratocystis fimbriata*. Its role as a carrot phytoalexin has been disputed, but it is now generally accepted that it is produced by the higher plant and not by the fungus in this interaction (cf. DEVERALL, 1976).

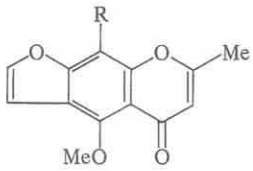
Chromones are isomeric with coumarins but fewer are known and most occur in only one or two plant sources. Eugenin (XLVII) is an example of a simple 2-methyl-chromone, present in *Eugenia aromatica* (Myrtaceae). Simple 5,7-dihydroxychromones unsubstituted in the 2-position e.g., (XLVIII), have been reported in several plants, including the peanut *Arachis hypogaea* (PENDSE et al., 1973), but recent evidence indicates that they are not actually plant products, but are formed as artefacts from flavanone glycosides present during the drying of the plant tissue



Eugenin
(XLVII)



5,7–Dihydroxychromone
(XLVIII)



R = OMe, Khellin (XLIX)
R = H, Visnagin (L)

before extraction (STOCKER and POHL, 1976). The only plant chromone unsubstituted at position 2 seems to be an unusual 3-ethylchromone which has been identified as a phytoalexin of the sweet pea, *Lathyrus odoratus* and *L. hirsutus* produced in the seed or leaf in response to fungal infection (ROBESON, D. and HARBORNE, J.B., unpublished results). Finally, mention must be made of furochromones, such as khellin (XLIX) and visnagin (L), which occur in fruits of *Ammi visnaga* (Umbelliferae), because of their pharmaceutical importance in the treatment of heart disease.

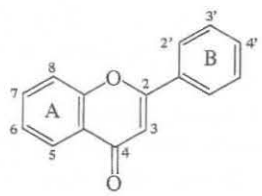
2.3 Flavonoids

2.3.1 The Common Flavonoids

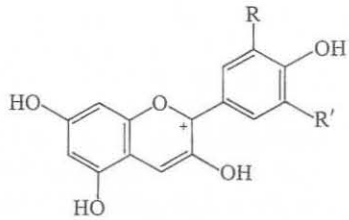
A large number of naturally occurring flavonoids have been described; according to a recent estimate (HARBORNE et al., 1975), there are about 2000 known structures. In spite of a bewildering array of structural variation, some simple patterns can be discerned. All are based on the same C_{15} skeleton of flavone (LI) and are formed by the same pathway from malonate units condensing with a phenylalanine-derived C_6-C_3 precursor.

Flavonoids are conveniently divided into some twelve classes, according to the oxidation level of the central pyran ring (Table 2). Most of these classes will be mentioned here but emphasis will be given to the three more important and widespread classes – the anthocyanins, flavones, and flavonols. It should be observed (Table 2) that the different classes vary in their biological properties; many (e.g., anthocyanins) are strongly pigmented but others (e.g., flavanones) are completely colourless. It is among the colourless flavonoids that compounds with significant physiological activity have been reported.

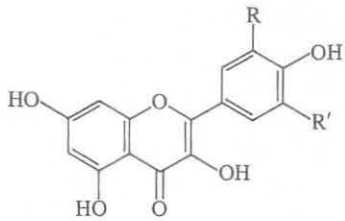
Of the several hundred flavonoid aglycones that have been isolated from plants only eight of these occur widely. One or other of these eight structures may be expected to be present in the hydrolysed extract of any higher plant studied.



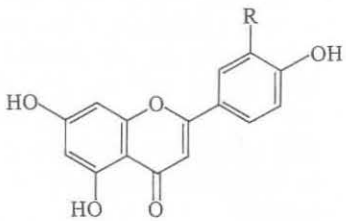
Flavone
(LI)



Pelargonidin, R = R′ = H (LII)
Cyanidin, R = OH, R′ = H (LIII)
Delphinidin, R = R′ = OH (LIV)



Kaempferol, R = R′ = H (LV)
Quercetin, R = OH, R′ = H (LVI)
Myricetin, R = R′ = OH (LVII)



Apigenin, R = H (LVIII)
Luteolin, R = OH (LIX)

Table 2. The major known classes of flavonoid

Class	No. of known structures	Biological properties
Anthocyanins	250	Red to blue pigments
Chalcones	60	Yellow pigments
Aurones	20	
Flavones	350	Cream pigments in flowers; feeding repellents (?) in leaves
Flavonols	350	
Flavanones	150	Some have bitter tastes
Dihydrochalcones	10	
Proanthocyanidins	50	Astringent substances, some with tanning ability
Catechins	20	
Flavan-3,4-diols	20	
Biflavonoids	65	None known
Isoflavonoids	150	Oestrogenic and fungitoxic

All eight of these flavonoids [(LII) to (LIX)] have similar hydroxylation in the A-ring and differ mainly in the oxidation level of the central pyran nucleus and in the number of hydroxyl groups in the B-ring: one, two, or three. All these common structures occur naturally in water soluble form as *O*-glycosides (see Sect. 3.2). Three are anthocyanidins: the scarlet-coloured pelargonidin (LII), the crimson cyanidin (LIII), and the mauve delphinidin (LIV). These three pigments or their simple methyl ethers occur very widely in plants, being particularly abun-

dant in coloured flowers and fruits. Within the angiosperms, they are almost ubiquitous, but are replaced in one group of families in the order Centrospermae (e.g., in beetroot, *Beta vulgaris*, Chenopodiaceae) by a different class of purple pigment, betacyanin, derived biosynthetically from the aromatic amino acid L-DOPA. Pelargonidin and delphinidin occur especially frequently in cyanic flowers, their synthesis being correlated with natural selection by animal pollinators for bright scarlet colours (hummingbirds) and deep blue colours (bees) respectively. By contrast, pigmented leaves normally contain cyanidin, the other two anthocyanidins being relatively rarely found in vegetative tissue.

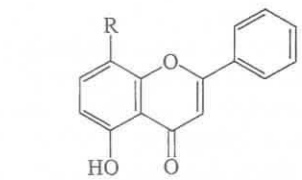
The flavonols corresponding in structure to the three main anthocyanidins are kaempferol (LV), quercetin (LVI) and myricetin (LVII), in order of increasing B-ring hydroxylation. While these flavonols usually accompany the anthocyanidins in flowers where they have an important role as co-pigments, they occur even more frequently, indeed almost universally, in plant leaves. A leaf survey of over 1000 angiosperms showed, for example, that 48% of species had kaempferol, 56% quercetin and 10% myricetin (SWAIN and BATE-SMITH, 1962). In all, over 60% of the sample had one or other of the three flavonols. While kaempferol and quercetin occur throughout the angiosperms, myricetin is more restricted in its occurrence, being present mainly in leaves of woody plants, in association with tannins.

Flavones lack the 3-hydroxyl group present in flavonols and anthocyanidins. Only two structures are common: apigenin (LVIII) and luteolin (LIX). These two flavones, besides occurring with flavonols, are also found on their own in many herbaceous plants, there being a tendency for them to replace flavonols in more specialised angiosperm families. Flavones, unlike the other common flavonoids, are frequently found in *C*-glycosidic combinations and are thus often reported as their *C*-glycosidic derivatives, vitexin (8-*C*-glucosylapigenin) and orientin (8-*C*-glucosyl-luteolin). They also occur widely in the more usual *O*-glycosidic combinations.

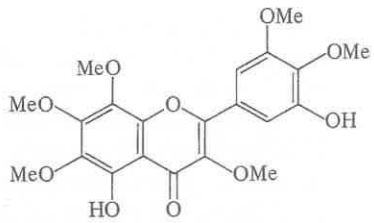
Tricetin, the flavone corresponding in structure to myricetin or delphinidin, is known, but so far appears to be of rare occurrence. It has been isolated from only a few plants, such as *Lathyrus pratensis* (Leguminosae), *Thuja occidentalis* (Cupressaceae) and *Lachenalia uniflora* (Liliaceae) (WILLIAMS et al., 1976). A methylated derivative of tricetin, namely tricin, is, however, more regular in occurrence, being particularly common as a constituent of grasses. Another peculiarity of flavones is their ability to link together by carbon–carbon bonds to form dimers. Over sixty biflavones are known, one of the commonest being amentoflavone or 3′,8″-biapigenin. These substances are largely restricted to and are typical of gymnosperm tissues, but they do occasionally occur elsewhere in the plant kingdom (GEIGER and QUINN, 1975).

2.3.2 Variations in Hydroxylation Pattern

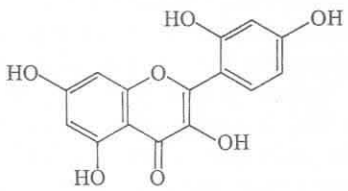
Many flavonoids differing in hydroxylation pattern from the eight common structures are known, but the great majority are of limited occurrence in nature. Some typical examples are compounds (LX)–(LXIX). The range extends from flavone itself and simple mono- and dihydroxyflavones (e.g., LX, LXI) which occur exclusively in farina on leaves of *Primula* species to compounds such as digicitrin



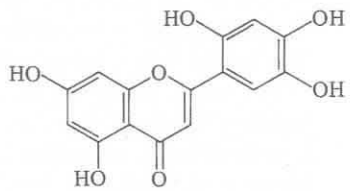
5–Hydroxyflavone, R = H (LX)
5,8–Dihydroxyflavone, R = OH (LXI)



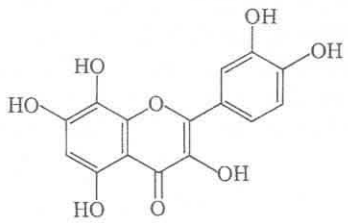
Digicitrin
(LXII)



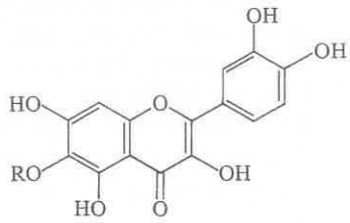
Morin
(LXIII)



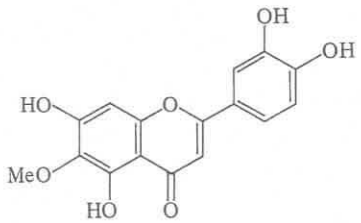
Isoetin
(LXIV)



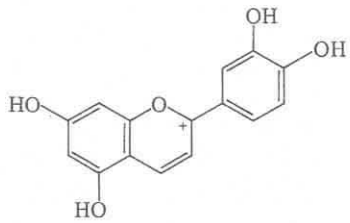
Gossypetin
(LXV)



Quercetagetin, R = H (LXVI)
Patuletin, R = Me (LXVII)



6–Methoxyluteolin
(LXVIII)



Luteolinidin
(LXIX)

(LXII) from the foxglove, which has eight hydroxyl groups, seven of which are protected by methylation. Most of the rarer flavonoids are closely related to one or other of the eight common structures, being formed from them by insertion of an extra hydroxyl function in an otherwise unoccupied site in the flavonoid nucleus. Thus, morin (LXIII) is derived from kaempferol by the insertion of an

extra hydroxyl in the 2′-position. This substance occurs in *Morus alba* leaves and is one of two flavonoids, the other being quercetin 3-glucoside, which contributes to the selective feeding of silkworm larvae on mulberry plants.

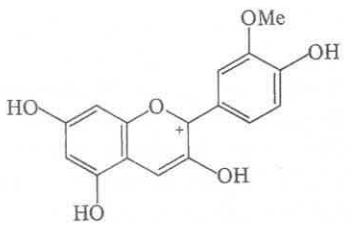
Similar insertion of a 2′-hydroxyl into luteolin (LIX) gives the flavone isoetin (LXIV) which has recently been identified as a yellow flower pigment in several members of the Cichorieae (Compositae) (HARBORNE, 1978 b). Yellow flower colour of a flavonoid nature is, however, more frequently based on the substitution of an extra hydroxyl group into the A-ring. Introduction of a hydroxyl at the 6- or 8-positions causes a significant shift in colour and such compounds, e.g., gossypetin (LXV) and quercetagetin (LXVI), are yellow instead of pale cream, as in quercetin (LVI). These two yellow pigments contribute colour to such well-known plants as the primrose, *Primula vulgaris*, cotton flower, *Gossypium hirsutum*, and corn marigold, *Chrysanthemum segetum*. In some plants, such as the composite *Rudbeckia hirta*, these yellow pigments have a special function because of their strong absorbance in the UV region, in that they can act as honey guides to insect pollinators. Patuletin (LXVII), a methyl ether of quercetagetin, for example, is differentially distributed in the inner part of the ray flower of *Rudbeckia*, while carotenoid pigments, which only reflect light in the UV, are evenly distributed over the whole ray (THOMPSON et al., 1972). Flavones [e.g., (LXVIII)] and flavonols with extra oxygen functions in the A-ring also occur regularly in leaves of angiosperms, particularly in herbaceous plants.

Other rarer flavonoids are those in which a particular hydroxyl function found in the common structures is removed rather than added to. One such example is luteolinidin (LXIX) which lacks the 3-hydroxyl group present in the common anthocyanidins and is referred to as a 3-desoxyanthocyanidin. Such pigments are rare in nature, but where they do occur, as in New World gesnerads, their synthesis seems to be correlated with hummingbird pollination and natural selection for scarlet or orange flower colour (HARBORNE, 1967 b).

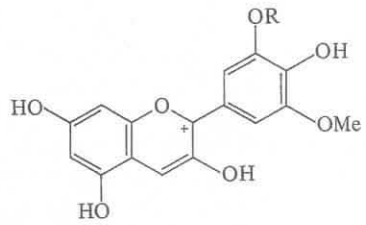
2.3.3 Methylated Flavonoids

The most common flavonoid *O*-methyl ethers are peonidin (LXX), petunidin (LXXI), and malvidin (LXXII), which are derived from cyanidin or delphinidin by methylation of the hydroxyl groups in the B-ring. Peonidin was first isolated from peony flowers, petunidin from *Petunia* and malvidin from *Malva*, but they are by no means restricted to their original plant sources. All three are actually widely present in plant tissues with cyanic colour. Petunidin and malvidin occur, for example, with delphinidin in grapes and the colour of genuine red wines is derived from these pigments.

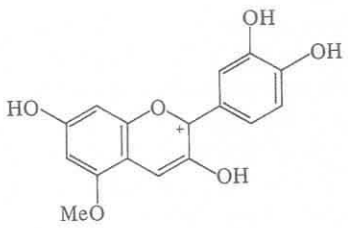
Very rarely, methylation may affect the hydroxyl groups of the A-ring of anthocyanidins, and two series are known with 5-or 7-*O*-methylation. 5-Methyl ethers of delphinidin, petunidin, and malvidin have been found exclusively in Plumbaginaceae, e.g., in the light blue corollas of *Plumbago capensis*, while 5-*O*-methylcyanidin (LXXIII) has recently been reported in *Egeria* (Elodeaceae) (MOMOSE et al., 1977). The 7-methyl ethers of peonidin and malvidin are called rosinidin and hirsutidin (LXXIV), respectively, and are likewise very restricted in their distribution. They only occur to any extent in one plant family, the Primulaceae, and here only in *Primula* and *Dionysia* species.



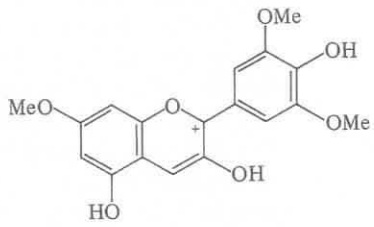
Peonidin
(LXX)



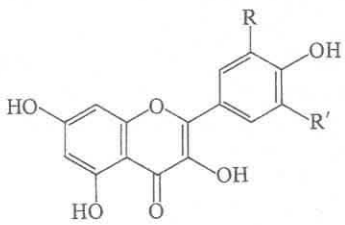
Petunidin, R = H (LXXI)
Malvidin, R = Me (LXXII)



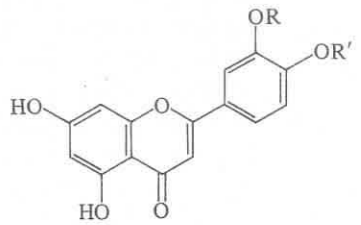
5–Methylcyanidin
(LXXIII)



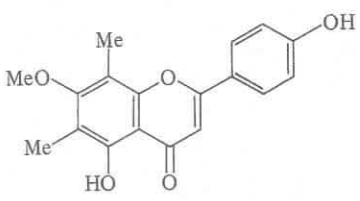
Hirsutidin
(LXXIV)



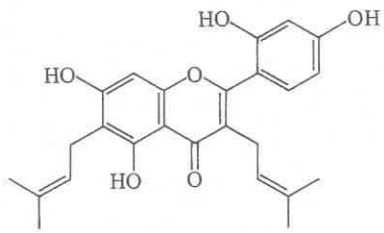
Isorhamnetin, R = OMe, R′ = H (LXXV)
Larycitrin, R = OMe, R′ = OH (LXXVI)
Syringetin, R = R′ = OMe (LXXVII)



Chrysoeriol, R = Me, R′ = H (LXXVIII)
Diosmetin, R = H, R′ = Me (LXXIX)



Sideroxylin
(LXXX)



Mulberrin
(LXXXI)

The three flavonol methyl ethers corresponding in structure to peonidin, petunidin, and malvidin are isorhamnetin (LXXV), larycitrin (LXXVI), and syringetin (LXXVII). At one time, they were thought to be relatively rare, but recent evidence suggests that they are in fact quite common. Isorhamnetin (LXXV) is now known to occur in leaves and petals of many plants and is also rather common as a pollen constituent. On the other hand, syringetin was not known as a natural product until its identification in *Lathyrus pratensis* flowers twelve years ago (HARBORNE, 1965). However, it has subsequently been found in a range of other dicotyledons, e.g. in *Philydrum* (Philydraceae) (BOHM and COLLINS, 1975) and also in monocotyledons, in *Elegia* (Restionaceae) and *Hedychium* (Zingiberaceae) (WILLIAMS and HARBORNE, 1977).

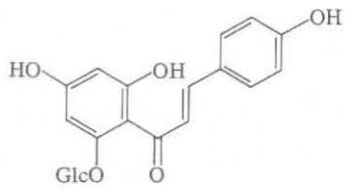
There are many other flavonol methyl ethers of more restricted natural occurrence. In the case of quercetin (LVI), all possible five monomethyl ethers are known, while at least seven isomeric dimethyl, two trimethyl, and three tetramethyl ethers have been described (GOTTLIEB, 1975). The frequency of methylation at the different hydroxyl groups of flavonols varies with their chemical reactivity, and while the 3-, 7- and 3′-hydroxyls often carry methyl substituents, 4′-methylation is less common and 5-methylation quite rare. Furthermore, if extra hydroxyl groups are substituted in the nucleus, e.g., in the 6-position, to give quercetagetin (LXVI), this extra hydroxyl is often methylated at the same time, e.g., giving compounds such as patuletin (LXVII). In the case of flavones, the most common methyl ether is chrysoeriol (luteolin 3′-methyl ether) (LXXVIII) but diosmetin (luteolin 4′-methyl ether) (LXXIX) and acacetin (apigenin 4′-methyl ether) are not uncommon, since both are known from a range of plants.

Methylation in the flavonoid series masks the reactive phenolic groups, at the same time changing the physical properties significantly by introducing lipid solubility into molecules which are otherwise hydrophilic. While substances with one *O*-methyl group (e.g., peonidin, isorhamnetin, chrysoeriol) usually occur in glycosidic combination in the water-soluble vacuolar fraction of the leaf, those with two or more methyl groups [e.g., digicitrin (LXII)] are often present without sugar attachment in a lipid-soluble fraction. The location of such methylated flavonols and flavones within the leaf has not been fully explored, but lipid solubility would suggest that they are either in the cytoplasm or in the wax of the leaf surface. Indeed, highly methylated derivatives have been isolated from leaf waxes in some plants, e.g., in *Eucalyptus*, and from bud exudates of *Populus* and *Alnus*. One structure from *Eucalyptus* leaf wax is sideroxylin (LXXX), which is unusual in being *C*-methylated as well as *O*-methylated.

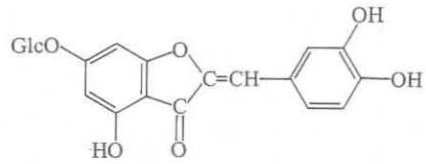
Finally, there is another structural feature in the flavonoid series which has the same effect as methylation on solubility: this is attachment of C_5 isoprenoid residues. One such compound is mulberrin (LXXXI), which is present in the bark of *Morus alba*. About 60 such structures have been listed, some having up to six C_5 residues attached (VENKATARAMAN, 1975).

2.3.4 Chalcones and Aurones

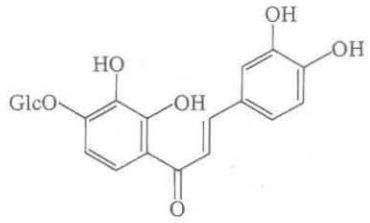
Chalcones and aurones are of principal biological interest because they contribute to yellow flower colour in a number of plants, especially members of the Compositae. Nevertheless, they do occur as colouring matters in other tissues, for example



Isosalipurposide
(LXXXII)



Aureusin
(LXXXIII)



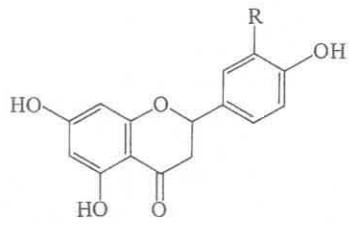
Marein
(LXXXIV)

in heartwoods and seeds of certain legumes. They are readily detected as flower pigments by the fact that when yellow petals are fumed with the alkaline vapour of a lighted cigar or of a bottle of ammonia, there is a dramatic colour change to orange or red. Because of this colour response, which is not shown by the more common yellow carotenoid pigments, these flavonoids are known as anthochlor pigments.

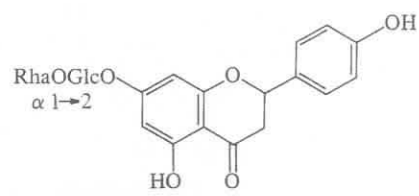
Such anthochlors may be the only class of yellow pigment in certain flowers. For example, the chalcone isosalipurposide (LXXXII) is the sole yellow colouring matter of yellow carnations, while the aurone aureusidin, occurring as the 6-glucoside aureusin (LXXXIII), is the major yellow pigment in the snapdragon, *Antirrhinum majus* (Scrophulariaceae). Anthochlors also occur regularly in yellow flowers accompanying lipid-soluble carotenoids. In such cases, the two classes of pigment may be differentially distributed in the flower tissue in such a way that the carotenoid is spread over the whole flower and acts as a general attractant to an insect visitor, while the yellow chalcone is present only in the inner ray and provides a UV absorbing honey guide to the centre of the flower. This happens in *Coreopsis bigelovii*, where the chalcone involved is marein (LXXXIV).

2.3.5 Flavanones and Dihydroflavonols

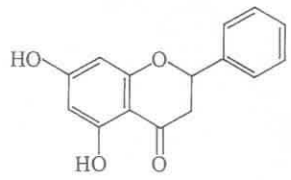
These two classes of flavonoid are simple reduction products of flavones and flavonols, respectively, and in terms of biosynthesis are formed first and then undergo oxidation in the 2,3-position to give rise to the more highly oxidised compounds. As biosynthetic intermediates, they are presumably universally present as trace constituents, but do not usually accumulate in quantity in leaf or flower tissue. Nevertheless, the two flavanones, naringenin (LXXXV) and eriodictyol (LXXXVI), corresponding in structure to apigenin and luteolin, have been recorded with some frequency in plants. They occur in glycosidic form and one particular



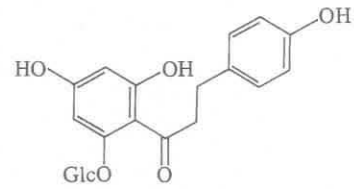
Naringenin, R = H (LXXXV)
Eriodictyol, R = OH (LXXXVI)



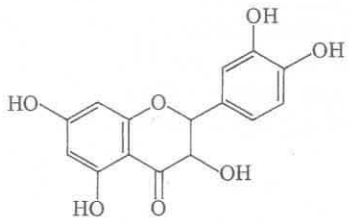
Naringin
(LXXXVII)



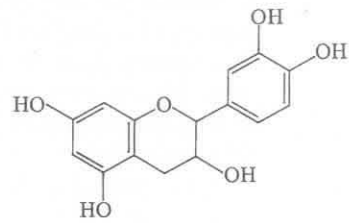
Pinocembrin
(LXXXVIII)



Phloridzin
(LXXXIX)



Dihydroquercetin
(XC)



Catechin
(XCI)

series of flavanone glycosides occurring in *Citrus* fruit with the disaccharide neohesperidose attached to the 7-position as in naringin (LXXXVII) are of special interest because of their bitter taste to humans. Free naringenin has been reported to occur in dormant peach buds, where it acts as an antagonist to gibberellins (PHILLIPS, 1962). Flavanones which lack B-ring hydroxyls, e.g., pinocembrin (LXXXVIII), are also known.

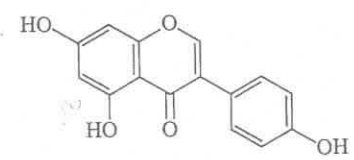
On ring opening, flavanones give rise to the corresponding chalcones and the two classes of flavonoids are closely inter-related. Indeed, there is an enzyme chalcone-flavanone isomerase present in plants which catalyses this inter-conversion. One type may also be formed from the other during extraction and work-up of plant extracts, unless special care is taken to use very mild conditions. Reduction of the chalcone formed by ring opening of a flavanone gives rise to yet another flavonoid class, the dihydrochalcone. Such compounds are quite rare in nature. However, one member of this group, phloridzin (LXXXIX), must be mentioned, because of its great pharmacological activity in man in producing glucosuria. Phloridzin occurs universally in the apple genus *Malus* and could function in leaves and roots as a herbivore-feeding deterrent because, like naringin, it has

a bitter taste. It is also a potent inhibitor of plant growth and may be responsible for the autotoxic effects of apple root extracts on the growth of apple seedlings (see RICE, 1974).

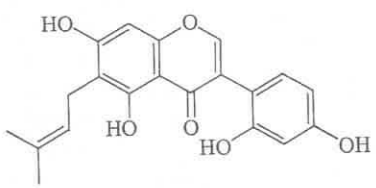
Dihydroflavonols corresponding in structure to the three common flavonols are all known, e.g., dihydroquercetin (XC), and are found most frequently in the free state in the heartwood of trees. They also occur occasionally in glycosidic form in leaf tissues. More highly reduced flavonoids, often co-occurring with dihydroflavonols, are the related flavan-3,4-diols and flavan-3-ols, where the 4-carbonyl is successively reduced to OH and H. Flavan-3-ols are also known as catechins and may have significant biological activity. While their main occurrence is in wood tissue, catechins also occur occasionally in leaves. For example, catechin (XCI) and its galloyl esters occur in high concentration in fresh leaves of *Camellia sinensis* and their oxidation products, formed during leaf fermentation, are responsible for much of the taste, flavour, and colour of tea. Catechins are also important as precursors of condensed tannins or proanthocyanidins (see Sect. 3.6).

2.3.6 Isoflavones and Isoflavonoids

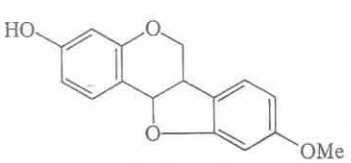
Isoflavones such as genistein (XCII) are isomeric with the flavones (e.g., apigenin) and are derived biosynthetically from the same flavanone intermediate by aryl migration (see Sect. 4). They appear to be more restricted in their natural distribution than flavones, since they have only been found regularly in one subfamily of the Leguminosae, the Papilionoideae. There are, however, occasional records in Amaranthaceae (*Iresine*), Iridaceae (*Iris*), Myristicaceae (*Myristica*), Moraceae (*Maclura*), and Rosaceae (*Prunus*). This suggests they could be more widespread and, since there is no satisfactory method of screening plant tissues for their presence, our present knowledge of their natural occurrence is certainly imperfect.



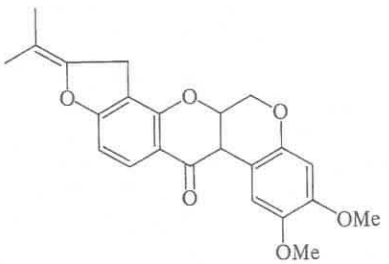
Genistein
(XCII)



Luteone
(XCIII)



Medicarpin
(XCIV)



Rotenone
(XCV)

As a group, isoflavones and their derivatives are significantly more biologically active than the corresponding flavones. While simple isoflavones may not be highly active as such, they can become active after structural modification. 6-Isopentenylgenistein, for example, together with the 2′-hydroxy derivative, luteone (XCIII), is significantly antifungal in its occurrence on leaf surfaces of *Lupinus* species (HARBORNE et al., 1976). More regular fungitoxic activity is displayed by reduced derivatives, isoflavans and pterocarpans such as medicarpin (XCIV), which are formed as phytoalexins in many legumes. Reduction in vivo of isoflavones, during ingestion by mammals, also leads to biological activity of an oestrogenic nature (see SHUTT, 1976). Finally, one other series of complex isoflavonoids containing isopentenyl substitution, must be mentioned. These are the rotenoids, e.g., rotenone (XCV), which are well known for their insecticidal activity. Rotenone is in fact highly toxic to all forms of life, since it inhibits respiration at an essential step in the terminal electron transport pathway, and blocks the catalysis of an $NADH_2$-dependent dehydrogenase.

2.4 Xanthones and Stilbenes

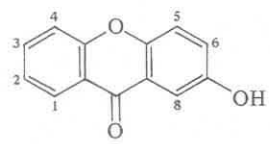
2.4.1 Xanthones

Although structurally related to the flavonoids and similar to them in chromatographic behaviour, the xanthones are much more restricted in their natural distribution. Indeed, practically all the known hydroxyxanthones have been obtained from either the Guttiferae or the Gentianaceae. They occur especially in the wood, roots, or leaves of these plants. More than 70 structures are known (CARPENTER et al., 1969), ranging from compounds with one hydroxyl (e.g., 7-hydroxyxanthone (XCVI)) to those with five hydroxyl (or methoxyl) substituents (e.g., 4,7-dimethoxy-1,3,8-trihydroxyxanthone (XCVII)). Although originally most xanthones reported were found in the free state in heartwood or root tissues, an increasing number of *O*-glycosides have been described more recently from leaves of *Gentiana* and *Swertia* species (HOSTETTMANN and WAGNER, 1977).

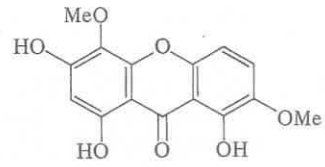
Mangiferin (XCVIII) is unique among the natural xanthones in having a much wider natural occurrence than any of the others: this is the *2-C*-glucoside of 1,3,6,7-tetrahydroxyxanthone and was first found in leaves of the mango tree *Mangifera indica*. It has since been found not only in ferns, e.g., in *Asplenium* (HARBORNE et al., 1973) but also sporadically in at least 15 angiosperm families (CARPENTER et al., 1969). More recent reports of its occurrence include *Heptaptera* (Umbelliferae) (HARBORNE, 1971), *Senecio* and *Dahlia* (Compositae) (GIANNASI, 1975) and *Polystachya* (Orchidaceae) (WILLIAMS, 1979).

2.4.2 Stilbenes

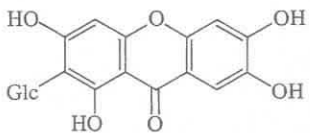
The most widespread natural stilbene is lunularic acid (XCIX), actually a dihydrostilbene, which occurs in all liverworts as a natural growth inhibitor, replacing abscisic acid, the dormancy hormone of most other plant groups. Lunularic acid is usually accompanied in liverworts by its decarboxylated derivative, lunularin (C), which is inactive as a hormone (GORHAM, 1977). The acid is assumed to have a



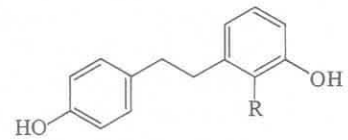
(XCVI)



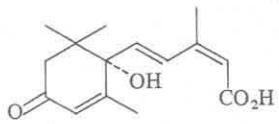
(XCVII)



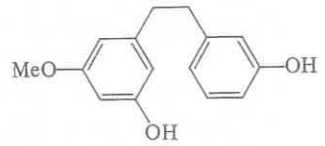
Mangiferin
(XCVIII)



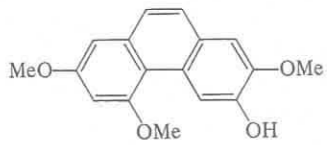
R = CO_2H, Lunularic acid (XCIX)
R = H, Lunularin (C)



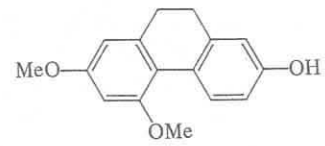
Abscisic acid
(CI)



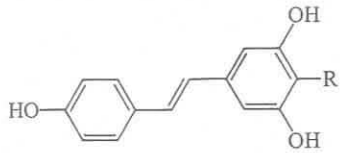
Batatasin III
(CII)



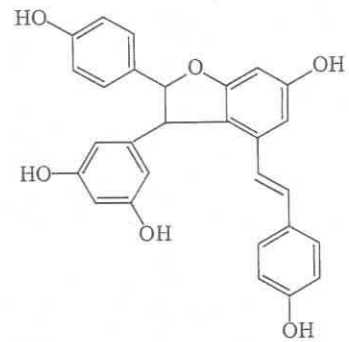
Batatasin I
(CIII)



Orchinol
(CIV)



R = H, Resveratrol (CV)
R = Isopentenyl (CVI)



e–Viniferin
(CVII)

similar hormonal role to abscisic acid (CI) and although not a sesquiterpene, it does have an analogous structure. Lunularic acid may also occur in some algae (PRYCE, 1972a, b), although some of the earlier reports of such occurrences have recently been questioned (GORHAM, 1977). This hormonal stilbene is otherwise absent from plants, although it does occur exceptionally in one angiosperm species, in *Hydrangea macrophylla*.

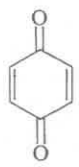
Another dihydrostilbene with physiological activity is batatasin III (CII), which is one of three phenolic growth inhibitors responsible for inducing dormancy in bulbils of the yam *Dioscorea batatis* (HASHIMOTO et al., 1974). A second compound, batatasin I, has been characterised as 6-hydroxy-2,4,7-trimethoxyphenanthrene (CIII) (LETCHER, 1973), a compound which could be derived biogenetically from a stilbene precursor. It is interesting (see below) that three related 9,10-dihydrophenanthrenes, e.g., orchinol (CIV), occur as antifungal agents in infected corms of *Orchis militaris* and *Loroglossum hircinum* (Orchidaceae) (FISCH et al., 1973).

Phenolics such as (CIII) with a phenanthrene skeleton may act as endogenous growth inhibitors in other plants besides the yam. It is significant that REISCH et al. (1973) have reported several similar phenanthrenes in rhizomes of *Tamus communis*, also Dioscoreaceae, and the same type of compound occurs in an unrelated dicot source in *Combretum* (Combretaceae) (LETCHER et al., 1972).

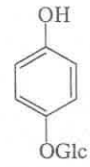
Most of the other known stilbenes have an unsaturated double bond between the benzene rings, typical members being pinosylvin (3,5-dihydroxy) in heartwood of *Pinus* and resveratrol (CV) in *Eucalyptus* trees (Myrtaceae). Hydroxystilbenes are of biological interest because of their antifungal properties and there is presumptive evidence that they protect the heartwoods of trees from fungal infections. This view of their antimicrobial activities has been confirmed by recent discoveries of resveratrol and its isopentenyl derivative (CVI) as phytoalexins produced in several legumes, e.g., *Arachis hypogaea* and *Trifolium*, in response to fungal attack (KEEN and INGHAM, 1976). Resveratrol has also been found as a phytoalexin in the unrelated *Vitis vinifera* (Vitaceae), where it also acts as a precursor of dimeric and trimeric derivatives, ε- and α-viniferin (CVII), which are more effective antifungal agents than the parent stilbene (LANGCAKE and PRYCE, 1977).

2.5 Quinones

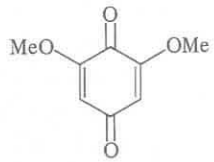
Most of the naturally occurring quinone pigments contain phenolic or methoxyl substituents and can thus be classified under the heading of plant polyphenols. However, even those quinones which do not have phenolic substitution (e.g., the plastoquinones) are related simply by reduction to the corresponding *p*-quinols and may actually occur in vivo in reduced form, with sugar attached to one of the quinol hydroxyls. Indeed, simple benzoquinones are highly reactive substances of considerable toxicity and do not normally occur in living tissues in any quantity. The parent substance, *p*-benzoquinone (CVIII), is used by a variety of arthropods in their defense secretions; the bombardier beetle in particular employs it in an explosive discharge of vapour. It is not surprising therefore, that benzoquinone only occurs in plants in reduced form as hydroquinone (1,4-dihydroxybenzene), as its glucoside, arbutin (CIX). The regular presence of hydroquinone in Ericaceae and Rosaceae has already been mentioned (Sect. 2.1).



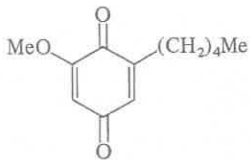
p–Benzoquinone
(CVIII)



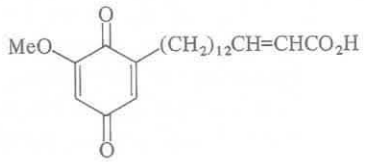
Arbutin
(CIX)



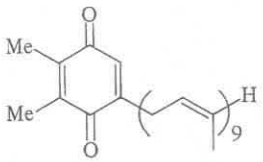
2,6–Dimethoxybenzoquinone
(CX)



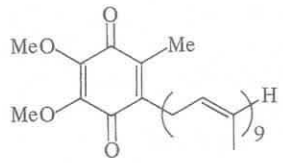
Primin
(CXI)



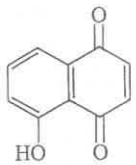
Sarcodontic acid
(CXII)



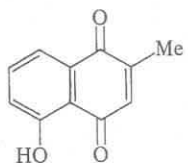
Plastoquinone–9
(CXIII)



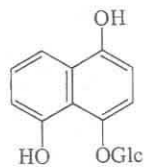
Ubiquinone–9
(CXIV)



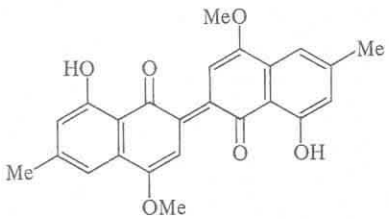
Juglone
(CXV)



Plumbagin
(CXVI)



(CXVII)



Diosindigo
(CXVIII)

The only simple substituted benzoquinone to occur widely in plants is the 2,6-dimethoxy derivative (CX), which occurs in wheat grains and also in a variety of other miscellaneous sources (see THOMSON, 1971). Again, it occurs in the reduced form as a monoglucoside and, in its occurrence in wheat germ, it appears to play a part in the germination process. The toxic effect of simple benzoquinones is well illustrated by the case of primin (6-methoxy-2-*n*-pentylbenzoquinone) (CXI), which is present in the glandular hairs of leaves of *Primula obconica* (Primulaceae), and is responsible for the allergic effects of this popular ornamental plant. Some people handling this *Primula* species develop skin dermatitis through the irritant properties of this quinone pigment.

Most of the other known simple benzoquinones (see THOMSON, 1971, 1976) occur in fungi, mainly in the Hyphomycetes and Basidiomycetes, where they may contribute to the colour of the fruiting bodies. Thus sarcodontic acid (CXII) is responsible for the intense sulphur yellow of the wood-rotting fungus *Sarcodontia setosa* found on old apple trees. More complex benzoquinones, with isoprenoid sidechains, also occur in plants but these are universally present, as carrier molecules in the electron transport chain. The two main classes are the plastoquinones, present in plant chloroplasts, and the ubiquinones, which are located in the mitochondria. In both groups, variation occurs in the length of the isoprenoid sidechain; two of the major components in plants are ubiquinone-9 (CXIII) and the corresponding plastoquinone-9 (CXIV).

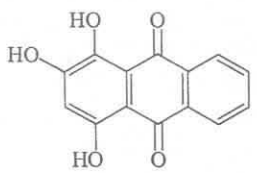
A second class of quinone pigment are those based on naphthalene – the naphthoquinones. Most of these are of higher plant origin and although intensely coloured are not often apparent as plant-colouring matters; they are often present in heartwood or bark, where their presence is masked. When present in living tissues, in leaves and roots, they are usually in colourless form and colour is produced only after extracts have been treated with acid to bring about hydrolysis of sugar linkages and oxidation of quinol to quinone. This is true of both juglone (CXV) of walnut and plumbagin (CXVI) of *Plumbago capensis*. Juglone (5-hydroxynaphthoquinone) is widely present in leaves and roots of members of the Juglandaceae but occurs as the 4-glucoside of the corresponding 1,4,5-trihydroxynaphthalene (CXVII). The quinone is released in the walnut during ripening of the fruit and is responsible for the yellow-brown staining of the skin caused by handling these nuts. Juglone (CXV) is also released in the soil around walnut trees, either as a root exudate or as washing from the leaves, and exerts an allelopathic effect in limiting the seed germination of certain annual plants which might otherwise grow in the immediate vicinity (RICE, 1974).

Plumbagin (CXVI), an orange pigment, is also present like juglone in bound form in plants. It is a more widespread compound, having been detected regularly in Plumbaginaceae, Droseraceae, and Ebenaceae and also once in Euphorbiaceae (*Pera ferruginea*). Like juglone, it is physiologically active; it has an irritating odour, affects the mucous membrane, stains the skin and produces blisters. Its allelopathic potential, however, does not seem to have been investigated.

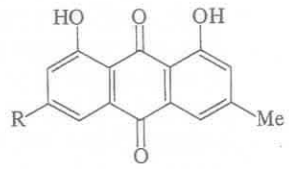
When present in heartwoods of trees, naphthoquinones occur free and a wide range of structures may be present together in any one given source. *Diospyros* (Ebenaceae) heartwoods are particularly rich, containing many derivatives of 7-methyljuglone in which molecules are linked together to form dimers, trimers,

and tetramers. One notable *Diospyros* dimer, named diosindigo (CXVIII), is actually blue in colour (THOMSON, 1976).

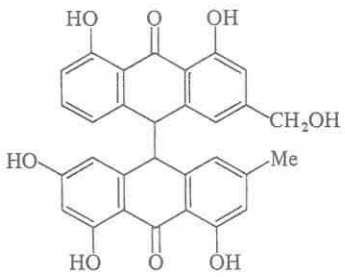
Much the largest group of natural quinones is the tricyclic anthraquinones and a wealth of structures are known with varying numbers of hydroxyl and other substituents. Over 200 occur in flowering plants, where they are found especially in the families: Leguminosae (*Cassia*), Liliaceae (*Aloe*), Polygonaceae (*Rheum, Rumex*), Rhamnaceae (*Rhamnus*), Rubiaceae and Scrophulariaceae (*Digitalis*). Members of the Rubiaceae (e.g., the madder plant, *Rubia tinctoria*) are characterised by having anthraquinones with phenolic groups in only one of the two benzene rings, e.g., alizarin (2,3-dihydroxy), anthragallol (2,3,4-trihydroxy) and purpurin (1,2,4-trihydroxy) (CXIX); these pigments have been used in the past for dyeing textiles. Other more common plant anthraquinones have hydroxyl substituents in both rings. Such compounds as emodin (1,3,8-trihydroxy-6-methyl) (CXX) and



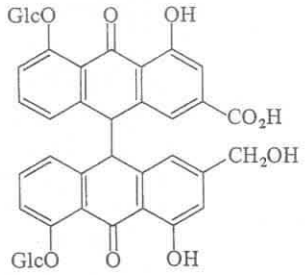
Purpurin
(CXIX)



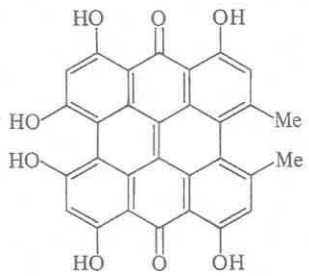
R = H, Chrysophanol (CXXI)
R = OH, Emodin (CXX)



Palmidin A
(CXXII)



Sennoside B
(CXXIII)



Hypericin
(CXXIV)

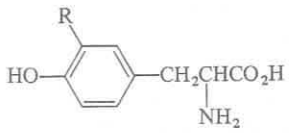
chrysophanol (1,8-dihydroxy-6-methyl) (CXXI) occur in several unrelated plant groups and also in fungi.

Emodin and chrysophanol, together with the related aloe-emodin (as emodin, but the 6-methyl is replaced by 6-CH_2OH) and rhein (1,8-dihydroxy-6-carboxy) occur characteristically in several plants used medicinally as a source of purgative drugs, i.e., the petioles of rhubarb, the rhizomes of *Rheum palmatum*, bark of buckthorn *Rhamnus cathartica*, and dried bark of *R. purshiana* (cascara). The free quinones, in fact, show little purgative activity and do not occur to any extent in these plants. They are present either in glycosidic combination (e.g., emodin as its 8-glucoside) or in reduced form (as anthrone) or dimeric reduced form (as bianthrone). Many of the naturally occurring bianthrones, which are the active purgative agents, are mixed dimers derived from more than one parent quinone. Rhubarb root, for example, contains palmidin A (CXXII), which is aloe-emodin: emodin bianthrone, while *Cassia* (senna pod) contains a bianthrone diglucoside, such as sennoside B (CXXIII). Among more highly condensed derivatives of emodin bianthrone is hypericin (CXXIV), a yellow photodynamic pigment widely present in leaves and stems of *Hypericum* species (Guttiferae). Finally, there are a variety of natural quinones which formally belong to other classes of natural product. Most of these are terpenoids but a few are flavonoids. The structural variation among such miscellaneous plant quinones has recently been reviewed by THOMSON (1979).

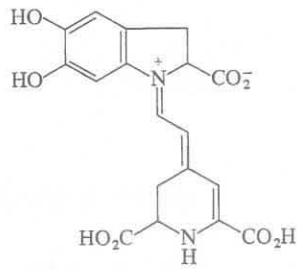
2.6 Miscellaneous Phenols

A number of plant substances belonging formally to other structural classes such as alkaloids and terpenoids also contain incidental phenolic substitutions. A familiar example among nitrogen compounds is the aromatic amino acid tyrosine (CXXV) which is of course ubiquitous in nature, both free and combined in protein. A number of related non-protein amino acids are also phenolic. There is, for example, 3,4-dihydroxyphenylalanine or DOPA (CXXVI), present in broad bean *Vicia faba* tissues and in seeds of a variety of other legumes (see BELL, Chap. 7 this vol.). Phenolic alkaloids are also regularly found in plants. Morphine, the pain-killing principle of the opium poppy, *Papaver somniferum*, has one phenolic hydroxyl, while betanidin (CXXVII), the purple pigment of beetroot and of other members of the Centrospermae, has two.

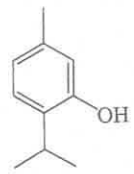
Among the various plant terpenoids, there are phenolic derivatives in most classes. Indeed, any alicyclic compound containing a six-membered carbocyclic ring with oxygen substituents is capable of undergoing aromatisation to yield a phenolic compound. A simple case in the monoterpene series is thymol (CXXVIII), a fairly common essential oil constituent, which has the same carbon skeleton as limonene. An example in the diterpene series is inuroyleanol (CXXIX), present in roots of *Inula royleana* (Compositae) (BHAT et al., 1975). An important group of pigments in the cotton plant is of the same type, the gossypols, the parent compound being gossypol (CXXX) itself. Another phenolic terpenoid pigment is 3-hydroxyisorenieratene (CXXXI), which is formally a carotenoid derived from β-carotene and has been isolated from *Streptomyces mediolani* (ARCAMONE



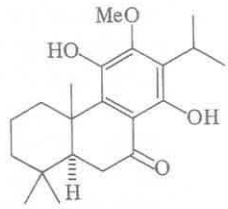
R = H, Tyrosine (CXXV)
R = OH, DOPA (CXXVI)



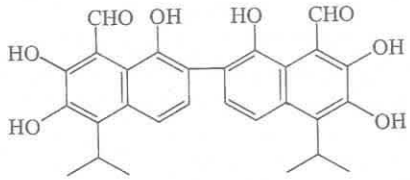
Betanidin
(CXXVII)



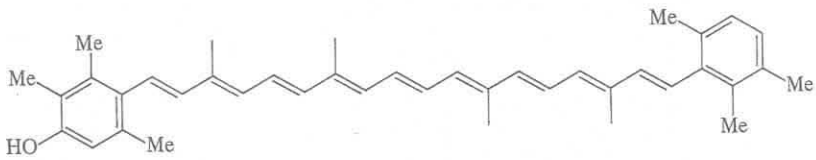
Thymol
(CXXVIII)



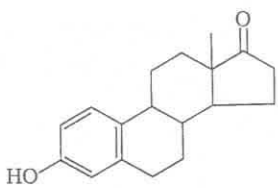
Inuroyleanol
(CXXIX)



Gossypol
(CXXX)



3–Hydroxyisorenieratene
(CXXXI)



Oestrone
(CXXXII)

et al., 1970). Finally, it is worth noting that mammalian female sex hormones, which are usually phenolic (e.g., oestrone, (CXXXII)) occur occasionally in plant tissues, e.g., in date palm seed (see HARBORNE, 1977a).

3 Phenolic Conjugates

3.1 Functional Significance of Conjugation

Phenolic compounds rarely occur in the free state in living plant tissue; they are practically always present in conjugated form. In the simplest instance, they are bound to sugar as β-D-glucopyranosides but a wide array of other bound forms are known. Undoubtedly one of the reasons why diphenols such as hydroquinone, catechol, or protocatechuic acid are bound to sugar is because of their potential toxicity in the free form to many forms of life. Simple phenols are caustic substances and well known to be potent antimicrobial agents. In medicine, the first successful antiseptic surgery was achieved following the use of phenol itself. The corresponding quinones are also toxic to most forms of life (see Sect. 2.5). The relative infrequency in plants of simple phenolic derivatives may well be related to their significant phytotoxicity. Where they do occur, they may become involved in allelopathic reactions between plants. Compounds such as hydroquinone or salicylic acid occur bound in the plant; they may be released into the environment from leaf or root in free form to exert an inhibitory effect on seed germination or plant growth in the surrounding soil (see HARBORNE, 1977a).

In the case of polyphenols such as the flavonoids, a bewildering array of conjugated forms is known. Glycosides are particularly abundant, but other forms of binding including sulphation or acylation are also possible. Reasons for the conjugation of flavonoids are complex but a major consequence of binding with sugar is their sequestration in the plant vacuole in a form and site where they cannot interfere with the vital enzymic processes of plant metabolism. It is significant here to note that when flavonoids occur in chloroplasts, as they apparently do in small amounts in almost all plants, they are always glycosidically bound. Although flavonols have often been considered to be quite harmless, there is increasing evidence of toxicity associated with some of these structures, particularly in the free state. Quercetin has recently been reported to be mutagenic (BJELDANES and CHANG, 1977) and has also been found to be an active inhibitor of cell membrane transport by its interference with the calcium-dependent ATPases (RACKER, 1975). In fact, free quercetin is capable of inhibiting a whole series of enzyme activities (see WAGNER, 1979).

One particularly important active site in polyphenols is the catechol nucleus, which has the ability to chelate metals and which may be directly involved in a number of biological reactions. For example, flavonoids with a 3′,4′-dihydroxyphenyl B-ring (e.g., quercetin) have a significant sparing effect in vitro on the destruction of auxin by IAA oxidase and thus if present at the growing point could indirectly stimulate growth in plants (see Sect. 6.1). Similarly in mammals, it has been found that catechol oestrogens inhibit elicited accumulation of hypothalamic cyclic AMP and hence may have a role as endogenous anti-oestrogens (PAUL

and SKOLNICK, 1977). In either case, masking of the catechol nucleus by *O*-glycosylation would undoubtedly have a profound effect on such biological activities.

Glycosylation of phenolic hydroxyl groups has a functional role in the case of those flavonoids which are coloured and occur in flower tissues. Also certain variations in the position of attachment of sugar to the flavonoid nucleus can produce significant shifts in visible colour. In the case of flavonoids of the leaf, it is more difficult to assign a role to conjugation but there are experiments which suggest the nature of the sugar present may determine whether a particular flavonoid is an effective feeding deterrent to insects and grazing animals. Glycosylation may thus be significant in relation to the ecological importance of flavonoids in plants (see HARBORNE, 1977a).

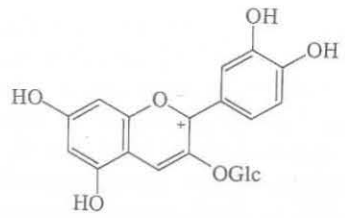
Finally, it may be observed that glycosylation also affects the physiological activities of flavonoids and other phenolics in plants. This is apparent from the experiments of STENLID (1970) and others; this subject is considered in more detail in Section 6.1.

3.2 Glycosidic Variation

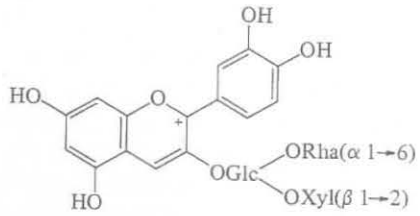
Most phenolics occur in plant leaf, stem, or flower in water-soluble form as glycoside and every plant that has been examined has yielded a water-soluble fraction in which the bulk of the low molecular weight phenolic material is present. The most common form of conjugate is phenolic glucoside, but combination with rutinose, as in the quercetin glycoside rutin, is also widespread. Hydroxycinnamic acids differ from most other phenols in occurring most frequently as quinic acid esters (see Sect. 3.3). When hydroxycinnamic acids occur with sugar, this is usually attached to the carboxylic acid grouping rather than to a phenolic residue. However, a caffeic acid derivative with both the 3-hydroxyl and the carboxyl group carrying glucose moieties has been described recently (IMPERATO, 1976).

Glycosidic variation is most marked among the flavonoids and in particular among the anthocyanins, flavonols, and flavones. In the case of the anthocyanins (glycosides of anthocyanidins), a comprehensive listing of known pigments in 1967 revealed the presence in plants of some 120 sugar derivatives (HARBORNE, 1967a); the number known today must be at least double this (see e.g., TIMBERLAKE and BRIDLE, 1975). With the common anthocyanidin cyanidin, about forty different glycosides are at present known. Although cyanidin 3-glucoside (CXXXIII) remains the most widely distributed anthocyanin, cyanidin derivatives with branched (CXXXIV) or linear (CXXXV) trisaccharides are by no means uncommon. The most highly glycosylated anthocyanin to date, however, is an acylated delphinidin derivative, isolated from flowers of *Lobelia erinus* (Lobeliaceae) (YOSHITAMA, 1977). This has five monosaccharide units: glucose and rhamnose (as the disaccharide rutinose) attached to the 3-position and glucose moieties separately linked at the 5-, 3′- and 5′-positions (CXXXVI). This pigment is also remarkable in being one of the first anthocyanins to be found with sugar substitution on the B-ring hydroxyls; glycosylation is much more commonly associated with only the 3- or the 3- and 5-positions.

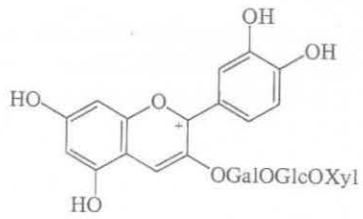
Quercetin is an extremely common plant polyphenol so it is not surprising that as many as 80 of its glycosides have been identified variously in plants (HAR-



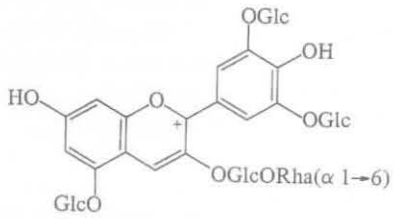
Cyanidin 3–glucoside
(CXXXIII)



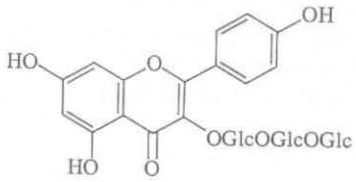
Cyanidin 3–(2^{G}–xylosylrutinoside)
(CXXXIV)



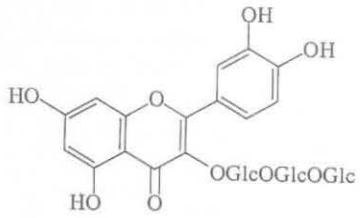
Cyanidin 3–(xylosylglucosylgalactoside)
(CXXXV)



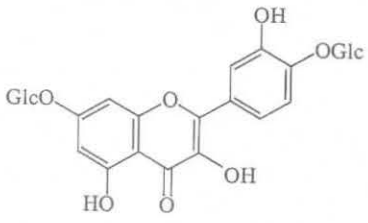
Delphinidin pentaglycoside
(CXXXVI)



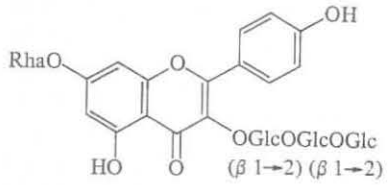
Kaempferol 3–triglucoside
(CXXXVII)



Quercetin 3–triglucoside
(CXXXVIII)



Quercetin 7,4′–diglucoside
(CXXXIX)



Kaempferol 3–sophorotrioside–7–
rhamnoside (CXL)

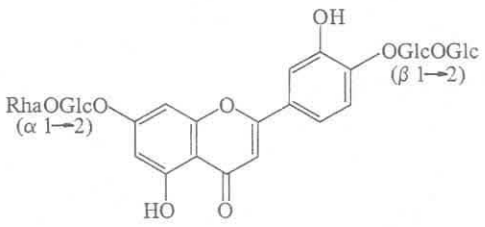
BORNE and WILLIAMS, 1975). Almost as many different glycosides of kaempferol and myricetin are also reported. An array of several glycosides is likely to be encountered in any given plant tissue. Pea plants, for example, contain at least four glycosides, the 3-triglucosides (CXXXVII), (CXXXVIII) of kaempferol and quercetin together with their *p*-coumaryl derivatives. These four substances have

been implicated by GALSTON (1969) in growth responses in *Pisum sativum*, while the quercetin acylated 3-triglucoside has been reported to be involved in tendril coiling in the same plant.

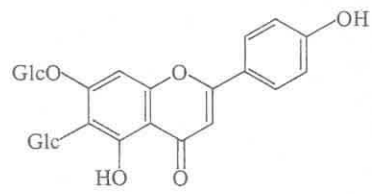
While quercetin 3-glucoside, cf. cyanidin 3-glucoside, is the commonest flavonol glycoside, the 3-rhamnoside, 3-galactoside, 3-glucuronide and 3-arabinoside of quercetin are also present relatively frequently in plants; all these simple monoglycosides co-occur, remarkably enough, in the skin of the common apple, *Malus pumila*. Rarer glycosidic types include the 3,4′- and 7,4′-diglucosides (CXXXIX) present in onion bulbs and the 3,3′-diglucoside present in horsechestnut seed. The most highly glycosylated flavonol glycoside remains the kaempferol derivative with four sugars (CXL) present in potato seed (see HARBORNE and WILLIAMS, 1975).

With the flavones, glycosidic complexity is compounded by the fact that sugars can be bound to phenolic hydroxyl groups and to the carbon nucleus of the aromatic ring at the 6- and/or 8-positions. Such *C*-glycosides are called glycoflavones and are capable of having *O*-sugars attached as well, either to one of the carbon-bound sugars or to a phenolic group. In case of simple flavone *O*-glycosides, the commonest are apigenin and luteolin 7-glucosides. Two examples of more highly substituted derivatives are luteolin 7-neohesperidoside-4′-sophoroside (CXLI) which occurs in the moss *Hedwigia ciliata* (ÖSTERDAHL and LINDBERG, 1977) and the acacetin pentaglycoside (CXLII) present in leaves of *Coptis japonica* (Ranunculaceae) (FUJIWARA et al., 1976). This latter compound has a branched tetrasaccharide in the 7-position and its structure is further complicated by the presence of no less than three acetyl groups attached to various sugar hydroxyls.

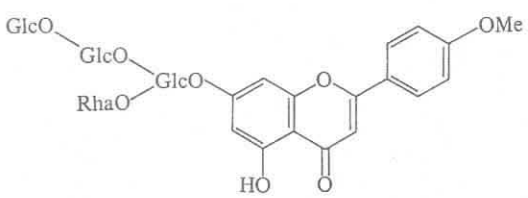
Among simple glycoflavones of plants is saponarin (CXLIII), a major compound of barley leaves, which is present not only in the vacuole but also in chloroplast preparations of this plant (SAUNDERS and MCCLURE, 1972). A more complex glycoflavone is vitexin 2″-sophoroside (CXLIV), reported in leaves of *Polygonatum odoratum* (Liliaceae) (MORITA et al., 1976). Particularly complex mix-



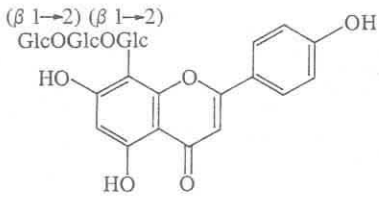
Luteolin 7–neohesperidoside–4′–sophoroside
(CXLI)



Saponarin
(CXLIII)



(CXLII)



Vitexin 2″–sophoroside
(CXLIV)

tures of *O*- and *C*-glycosides occur in grasses: no less than 26 compounds are present in oat leaves (POPOVICI et al., 1977) and 27 in barley leaves (FRÖST et al., 1977). The ability of Gramineae to accumulate unusual glycoflavones is illustrated by the isolation of the first *C*-galactoside, 8-*C*-galactosylapigenin, from *Briza media* (CASTLEDINE and HARBORNE, 1976). A recent review of plant glycoflavones is that of CHOPIN and BOUILLANT (1975).

3.3 Bound Forms of Hydroxycinnamic Acids

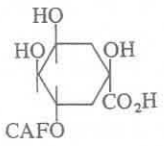
Hydroxycinnamic acids occur naturally in a much wider range of combined forms than any other group of plant phenols. While most frequently present as quinic acid esters, they may nevertheless occur conjugated with organic acids, sugars, amino compounds, lipids and terpenoids (Table 3). They may also be linked to other phenolic compounds and hydroxycinnamic acids are regularly found linked through sugar to flavonoid glycosides (see Sect. 2.3). In addition, as already men-

Table 3. Some simple esters of hydroxycinnamic acids in plants

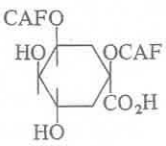
Class of compound	Ester group	Esterified acid and trivial name of conjugate
Cyclohexane carboxylic acid	Quinic acid	CAF: chlorogenic acid FER/COUM
	Shikimic acid	CAF/COUM
Cyclitol	*m*-Inositol	COUM
Sugar	Glucose	CAF/FER/COUM
	Rhamnose	CAF
	Fructose	COUM
	Gentiobiose	CAF/FER
	Rutinose	COUM
Organic acid	Tartaric acid	CAF: chicoric acid
	Malic acid	CAF: phaselic acid
Amino compounds	Dopaldehyde	CAF
	DOPA	CAF: clovamide
	Tryptamine	FER/COUM
	Putrescine	CAF: paucine FER/COUM
	Choline	SIN: sinapine FER
	Lupinine	COUM
Phenolics	Salicin	CAF: populoside
	3,4-Dihydroxyphenyl-lactic acid	CAF: rosmarinic acid
	Cyanidin 3-glucoside	COUM: hyacinthin
	Kaempferol 3-glucoside	COUM: tiliroside
Lipids	Hexacosylalcohol	FER
	Glycerol	CAF/COUM
Terpenoids	Borneol	COUM
	α-verbesinol	COUM
	Catalpol	COUM
	Queretaroic acid	CAF

Key: COUM = *p*-coumaric, CAF = caffeic, FER = ferulic, SIN = sinapic
In some cases, only a few of many known derivatives are listed

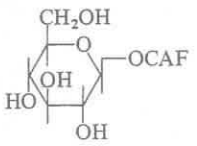
tioned (Sect. 2.2), ferulic acid can be associated in plants with protein and also with the cellulose and hemicellulose of plant cell walls. An account of the bound forms of hydroxycinnamic acids was given by HARBORNE (1964a). Since then, many more derivatives have been characterized, so that it is only possible to



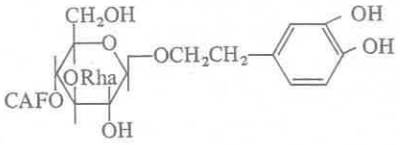
Chlorogenic acid
(CXLV)



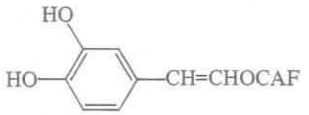
Cynarin
(CXLVI)



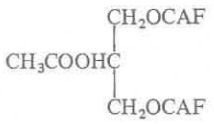
Caffeylglucose
(CXLVII)



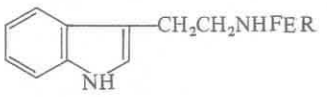
Acteoside
(CXLVIII)



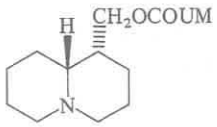
Caffeyldopaldehyde
(CXLIX)



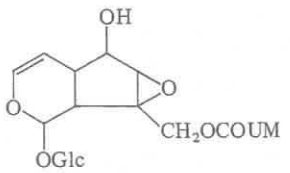
Lasiocarpin
(CL)



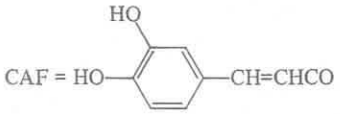
N–ferulyltryptamine
(CLI)

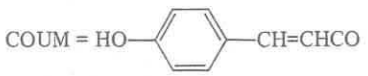


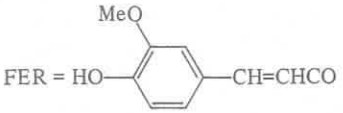
p–Coumaryl–lupinine
(CLII)



10–*p*–Coumarylcatalpol
(CLIII)







mention here a selection of recent structures involving the three most common acids, caffeic, ferulic, and *p*-coumaric.

Caffeic acid occurs most widely as the simple quinic acid ester, chlorogenic acid (CXLV), but dicaffeyl esters of quinic are also possible. Cynarin (CXLVI), for example, is the choleretic principle of the artichoke *Cynara scolymus* (PANIZZI and SCARPATI, 1965). Sugar derivatives of caffeic are also common, particularly the glucose ester (CXLVII); recently the rhamnose ester has been found in flowers of *Lantana hybrida* (Verbenaceae) (IMPERATO et al., 1975). Among the more complex sugar derivatives of caffeic acid is acteoside (CXLVIII) which contains both glucose and rhamnose and is found in flowers of *Syringa vulgaris* (Oleaceae) (BIRKOFER et al., 1968).

Among an increasing number of amino derivatives of caffeic acid to be reported in the last few years, the derivative with putrescine, $NH_2(CH_2)_4NH_2$, may be mentioned. This and related acylated amines appear to be especially associated with the reproductive tissues of angiosperms and may thus have a physiological role in flowering (TANGUY et al., 1978). Caffeic acid can also be linked to the 3-hydroxyl of the amino acid DOPA as in *Trifolium pratense* (YOSHIHARA et al., 1977) and to the aldehyde oxygen of dopaldahyde to give a yellow conjugate (CXLIX) recently detected in leaves of *Plectranthus caninus* (Labiatae) (ARIHARI et al., 1975). Several lipid-based derivatives of caffeic acid are also present in plants. The monoglyceride of caffeic acid is present in oat seeds and pineapple stems (TAKATA and SCHEUER, 1976) while a triglyceride, lasiocarpin C (CL), has been discovered in *Populus* bud extracts by ASAKAWI et al. (1977). In this and related compounds, glycerol is substituted in the 2-position by acetic acid, while the 1- and 3-positions are occupied by caffeic and/or *p*-coumaric acids.

Ferulic acid, like caffeic, has also been detected bound to lipid components and hexacosyl ferulate in needles of *Pinus roxburghii* (CHATTERJEE et al., 1977) is only one of a series of such hydrocarbon derivatives. Among other novel ferulyl conjugates is *N*-ferulyltryptamine (CLI) which is present in kernels of *Zea mays* (EHMANN, 1974). Conjugation with nitrogen compounds extends to the alkaloids and a variety of acylated alkaloids have been described (see SMITH, 1977). One recent example is *p*-coumaryl-lupinine (CLII) from *Lupinus luteus*, which occurs as such and also with rhamnose attached to the phenolic group of the *p*-coumaryl residue (MURAKOSHI et al., 1977).

Finally, mention should be made of the many hydroxycinnamyl residues which are linked to aliphatic hydroxyl groups among terpenoids (Table 3). *p*-Coumaric acid, for example, is acylating the monoterpene borneol in *Seseli mucronatum* (DUKHOVLINOVA et al., 1975), the monoterpene lactone catalpol (CLIII) in *Scutellaria altissima* (WEINGES et al., 1975) and the sesquiterpene alcohol, α-verbesinol in *Verbesina virginiana* (GARDNER et al., 1961).

3.4 Lipid-Soluble Derivatives

The phenolic compounds that occur in lipid-soluble plant extracts have been less well studied than those in water-soluble extracts, so that much less is known about them. One of the more unexpected discoveries of the last few years has been of water-soluble phenolics occurring frequently, to a small extent, in the

characteristically lipid-based environment of the plant chloroplast (see McCLURE, 1975). It is conceivable that lipid-soluble phenolics may well be found to be regular constituents of this and other cell organelles, e.g., in the cytoplasm, but so far this is an area for future investigation. The only situation where lipid-soluble phenols have been found regularly is in the waxy hydrocarbon coating of plant leaves. Even here, few detailed studies have been made of the phenolic substances present.

Lipid-soluble phenolics have been more fully characterised only in the more exceptional situations of bud exudates from angiosperms and gymnosperm trees, of the crystalline pigmented deposits on the under-surfaces of fern fronds and of the waxy farina present on *Primula* plants. In such instances, the most significant chemical feature is that of a variable number of phenolic groups carrying *O*-methyl substitution. *O*-Methylation thus appears to be an important process, at least in polyphenols, which masks the reactivity of the phenolic groups and at the same time significantly increases the lipid-solubility and volatility. With simple phenols, such as the hydroxyphenylpropenes, *O*-methylation or methylenedioxy ring formation is the rule rather than the exception and this makes the substances so volatile that they are not only readily extracted into ether but are also steam-volatile and appear with monoterpenes in the "essential oil" fraction. Such masked phenols as methyleugenol or myristicin are readily detected by insects and play a significant role, when present in flowers or leaf, in attracting or deterring insects to feed or oviposit on plants containing them (see HARBORNE, 1977a).

The importance of methylation for masking phenolic groups is further emphasised by the fact that polyhydroxy compounds are rarely found in any concentration in any of these lipid-soluble fractions. In the lipid-soluble farina of *Primulas*, flavone itself is, in fact, the major component. Trace amounts of mono- and dihydroxyflavones may accompany it, but only one trihydroxyflavone (5,8,2′-) has ever been found and this only occurs in three of several hundred known species (BOUILLANT et al., 1971). Similarly, in the bud exudates of *Betula* and *Populus* studied mainly by WOLLENWEBER (1976), the flavones and flavonols present rarely have more than one or two free hydroxyls. Typically present are kaempferol 3,7- and 3,4′-dimethyl ethers and various isomeric trimethyl ethers of quercetin, together with apigenin 7,4′-dimethyl ether and luteolin 7,3′,4′-trimethyl ether. More highly *O*-methylated derivatives may also be present, e.g. 3,7,3′,4′,5′-pentamethyl ether of myricetin. It is possible that the only situations where flavonols such as kaempferol, quercetin, and myricetin occur in the free state, without methylation or glycosylation, is in dying or dead plant tissue, especially in seed coats or in the heartwoods of trees.

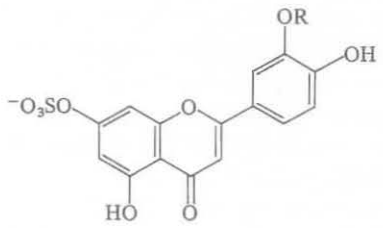
An alternative or additional means of introducing lipid-solubility into phenolic nuclei is by the attachment of isoprene residues. These are most frequently attached directly to the aromatic ring, but they may also be attached to a phenolic group. One example of such a compound is luteone, 6-isopentenyl-5,7,2′,4′-tetrahydroxy-isoflavone, which occurs in leaves of *Lupinus* (Leguminosae). While it occurs to some extent within the leaf, its main site is on the surface in the leaf wax. It is highly fungitoxic and occurs in sufficient amount to act as an important barrier to microbial infection in these plants. Isopentenyl substitution, besides conferring lipid solubility to luteone, considerably enhances its biological properties, since related isoflavones lacking this substituent have only one-tenth the fungitoxicity

exhibited by luteone (HARBORNE et al., 1976). Luteone is only one of some 400 phenolic compounds with isoprenoid substituents, most of them being coumarins (see Sect. 2.2).

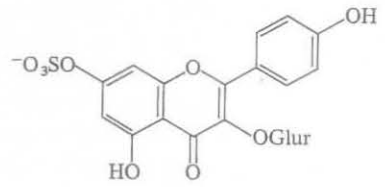
Finally with regard to lipid-soluble hydroxycinnamic acids, as mentioned in the previous section, these are present in many different tissues. Unlike the above phenolics, these derivatives actually have lipid components, including glycerol itself and long-chain hydrocarbon alcohols.

3.5 Sulphates

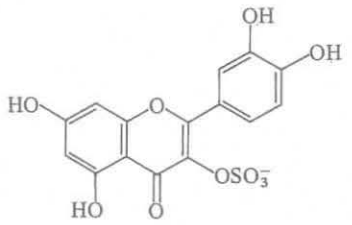
A recent discovery is that flavonoid sulphates are relatively widespread in the leaves of plants. Approximately 50 conjugates have been identified in over 200 species drawn from 20 families (HARBORNE, 1977b). Some of these flavone and flavonol sulphates contain sugar as well (CLIV)–(CLV), but the majority simply have one or more bisulphate groups directly attached to phenolic hydroxyls (CLVI)–



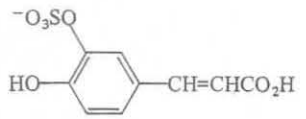
R = H, Luteolin 7–sulphate (CLVII)
R = Glc, Luteolin 7–sulphate 3′–glucoside (CLIV)
R = SO_3^-, Luteolin 7,3′disulphate (CLVIII)



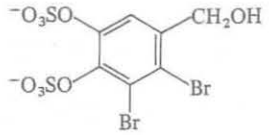
Kaempferol 3–glucuronide–7–sulphate (CLV)



Quercetin 3–sulphate (CLVI)



Caffeic acid 3–sulphate (CLX)



(CLIX)

(CLVIII). Such an attachment converts a neutral molecule to an anionic compound and has a dramatic effect in providing very considerable water and hence sap solubility. The direct effect of sulphation is thus similar to that of glycosylation and it is an interesting question why such an obviously alternative system of conjugation should have arisen in certain plants.

If sulphation is a simple alternative to glycosylation in plants, one might expect it to affect other classes of phenol. This is in fact so, and other phenolic conjugates of this type are being discovered quite regularly. One of the simplest is a sulphated catechol (CLIX) that has been found in the red alga *Polysiphonia* (see THOMSON, 1977). Other simple compounds include the 3-sulphates of caffeic acid (CLX) and gallic acid that have been detected, together with quercetin 3-sulphate (CLVI), in plants of the Polygonaceae. Yet another type has been found in the same family: the first reported anthraquinone sulphates. These occur in leaves of *Rumex pulcher* and include emodin 1(or 8)-glucosidesulphate and its dianthrone derivative (HARBORNE and MOHKTARI, 1977).

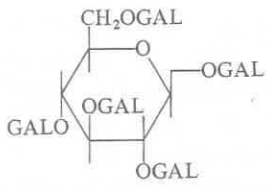
Another area where flavonoid sulphates occur regularly is in marine higher plants and especially in members of the order Fluviales. For example, eel grass, *Zostera marina*, contains all its flavone conjugated with sulphate, luteolin 7,3′-disulphate (CLVIII) being one of its characteristic constituents. In other Fluviales, e.g., turtle grass *Thalassia testudinum*, flavonoid sulphates appear to be replaced by simpler conjugates, since several isomers of chlorogenic acid containing sulphate occur in these plants (HARBORNE and WILLIAMS, 1976).

Finally, one other type of sulphated caffeic acid derivative has been noted, that based on the glucose ester, and such compounds have been noted in several ferns and in *Paspalum convexum* (Gramineae) (COOPER-DRIVER and SWAIN, 1975).

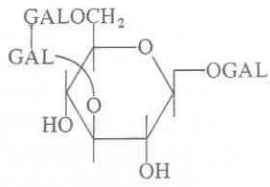
It is clear that conjugation of phenols with sulphate is a general phenomenon, not restricted to flavonoids, and may occur with a variety of phenolic substrates. Undoubtedly, more types will be found in future investigations. Their significance in plant metabolism is not yet clear, but their regular presence in water plants and also in halophytes (see HARBORNE, 1977a) suggests they may play a part in inorganic ion absorption, storage or metabolism. Apart from the covalent binding of sulphate, these phenolic derivatives also bind potassium ion and this may be as important as their anionic sequestration.

3.6 Polymers

One process which probably reduces the chance of phenolic compounds interfering with enzymic reactions in the cell is polymerisation. Undoubtedly, as the molecular weight of a phenolic compound increases, its transport within the cell is considerably diminished. The two main types of phenolic polymers in plants are the lignins and the tannins. Lignins are sequestered in the secondary layer of the cell wall in close association with a cellulose matrix, where the phenolic hydroxyl groups may be hydrogen bonded or even covalently linked to carbohydrate. The number of phenolic groups in lignin is low because many phenolic functions are lost as part of the polymerisation process and appear in lignin structure as ether linkages. It is interesting in this context that the three monomers of lignin (namely *p*-coumaryl, ferulyl, and sinapyl alcohols) only have a single *p*-hydroxyl group, i.e., lignins

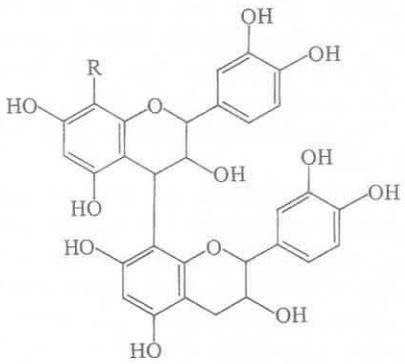


Pentagalloylglucose
(CLXI)



Corilagin
(CLXII)

GAL = galloyl



Procyanidin (CLXIII)
R = H, dimer
R = Flavan, trimer
R = further flavans, polymer

never incorporate the more reactive catechol-containing caffeyl residues. For a recent review of lignin biochemistry, see GROSS (1979).

In the case of the plant tannins, there is a big difference in molecular size between the hydrolysable and condensed tannins, the latter being of much higher molecular weight than the former. The two groups also differ in that hydrolysable tannins usually contain glucose as an integral part of their structures. Pentagalloylglucose (CLXI), the simplest hydrolysable tannin, is thus a conjugated form of gallic acid, even if the ratio of glucose to gallic acid is rather low. In other hydrolysable tannins (CLXII), glucose may be linked to phenolic groups to render the molecules more water-soluble and ensure their sequestration in the vacuole.

Condensed tannins (e.g., CLXIII) or proanthocyanidins (HASLAM, 1975) by contrast, appear never to be associated with sugar, although there have been occasional reports of glucose being present. The biological activity of condensed tannins is limited by their high molecular weight and their relative immobility. Nevertheless, the absence of any protective groups on the hydroxylated matrix of these polymers is probably the reason why they are so important in plant–animal interactions. When cell organisation is disrupted, e.g., when an insect bites into a leaf, these tannins readily complex with proteins, significantly reducing their enzymic functions or nutritive value in the process. It is interesting that no detoxification process is known in animals which is capable of dealing with these reactive polymers.

The ability of polymeric proanthocyanidins to interfere with growth processes in plants must be severely limited by their relative immobility. The degree of polymerisation is critical also for the ability to tan protein (see SWAIN, 1965) and the lower oligomers of flavolan have little tanning ability. This probably explains why FEUCHT and NACHIT (1977) have been able to observe growth-promoting effects in young shoots of *Prunus avium* by application of catechins and dimeric flavolans. These relatively low molecular weight substances presumably are able to have a sparing effect on IAA, without interfering at the same time with any of the vital processes of growth.

4 Biosynthesis and Metabolism of Phenolics

4.1 Carbon Pathway

4.1.1 Introduction

The biosynthetic origins of the carbon atoms in most classes of phenolic compounds are now well established, as a result of a variety of tracer studies applied to whole plants, organ cultures and tissue cultures. Some of the main pathways to the more common phenolic constituents are outlined in Figure 3. It should be emphasised that many classes may be formed by more than one route; this applies particularly to the quinone pigments, but is also becoming apparent for certain types of phenylpropanoid. The biosynthesis of phenolics has been extensively reviewed in recent years (see especially HAHLBROCK and GRISEBACH, 1975; HASLAM, 1974; HARBORNE, 1977c). Here only a summary of the main findings will be included.

4.1.2 Phenolic Acids

There are two possible routes to these benzoic acid derivatives. They may be formed by the β-oxidation of C_6-C_3 hydroxycinnamic acids, or more directly by aromatisation of shikimic acid or of a related cyclohexane derivative (*dotted line* in Fig. 3). The weight of evidence is in favour of the former route being the most important biosynthetically; thus extensive radiocarbon studies by TERASHIMA et al. (1975) of *p*-hydroxybenzoic acid synthesis in *Populus nigra* support the route via *p*-coumaric acid. The actual mechanism of the oxidation of the C_3 sidechain has been examined by FRENCH et al. (1976) in potato tuber tissues, and it does not appear to involve the coenzyme A ester, as might be expected in the normal β-oxidation pathway. Instead, the double bond is hydrated, there is cleavage of a two-carbon fragment and the aldehyde produced is then enzymically oxidized to carboxylic acid, the route being: $RCH=CHCO_2H \rightleftharpoons RCHOHCH_2CO_2H \rightleftharpoons RCHO \rightleftharpoons RCO_2H$. The enzymes involved are heat-labile and unstable and thus have not yet been studied in detail.

4.1.3 Hydroxycinnamic Esters and Hydroxycoumarins

The CoA esters of hydroxycinnamic acids have a central role in phenolic biosynthesis (ZENK, 1979) and these substances are known to be the essential intermediates

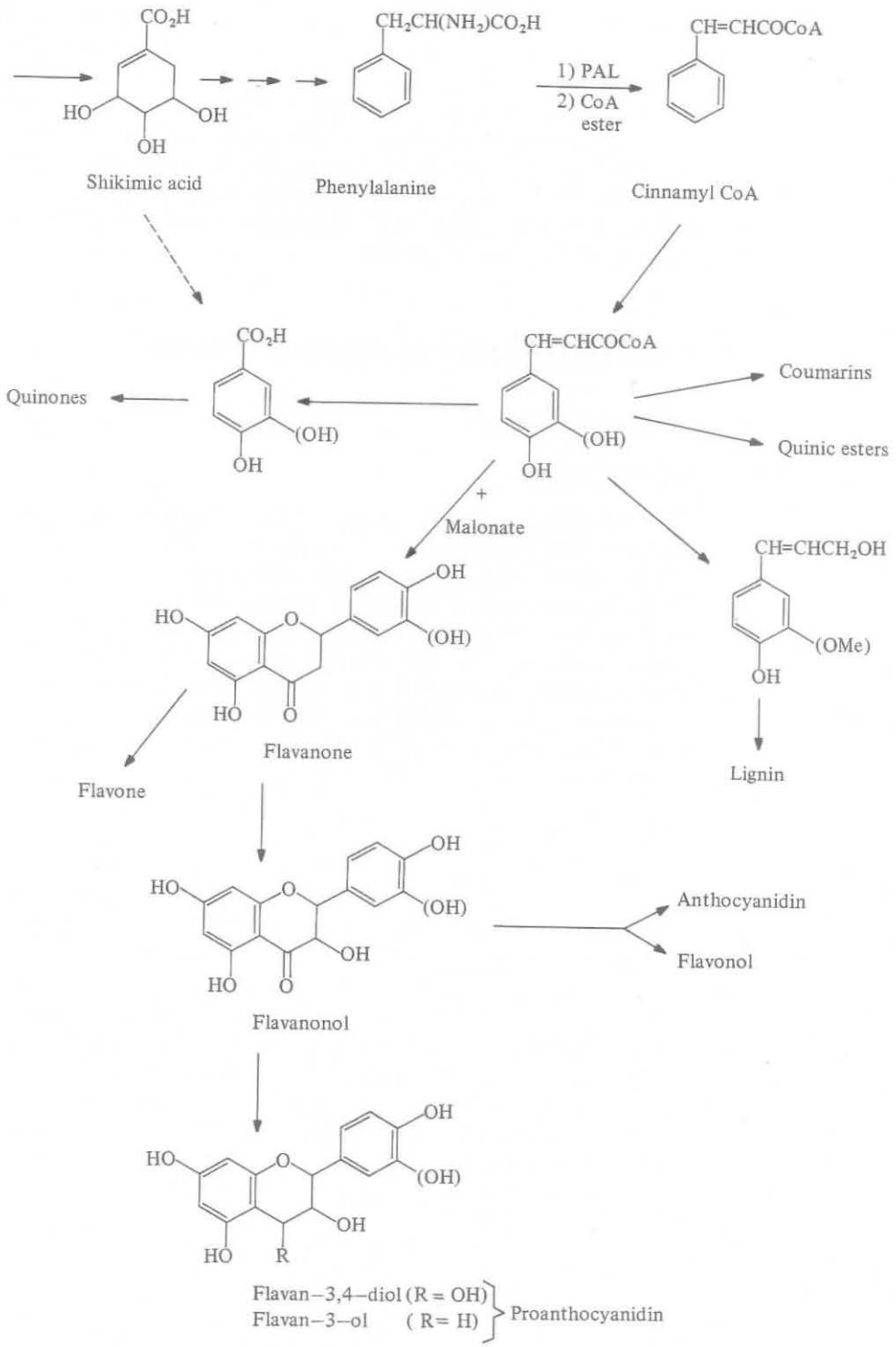


Fig. 3. Pathway of biosynthesis of flavonoids and related plant phenolics

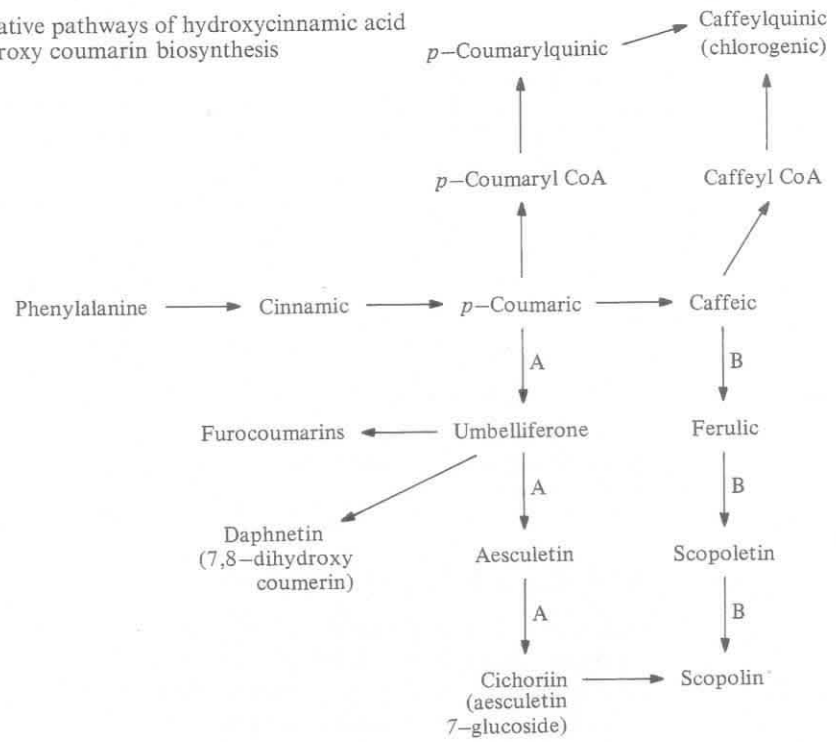


Fig. 4. Alternative pathways of hydroxycinnamic acid ester and hydroxy coumarin biosynthesis

in the production of the many and varied bound forms of *p*-coumaric, caffeic, and ferulic acids present in nature. There are still, however, some uncertainties in the actual pathways of biosynthesis. Two alternative routes, differing in the stage at which a second hydroxyl group is introduced into the phenyl ring, have been proposed for the synthesis of the well-known quinic ester, chlorogenic acid (Fig. 4). It is of course possible that both routes are used, depending on the availability of substrates at a particular site of synthesis. More work is needed to establish the relative importance of these alternative pathways.

The actual route to hydroxycoumarins in plants may also vary depending again on the stage at which the second hydroxyl is introduced into the phenylpropanoid intermediate. Feeding experiments in *Daphne odorata* and *Cichorium intybus* have established route A (Fig. 4) for aesculetin and cichoriin synthesis in these plants, while experiments in tobacco tissue culture show that scopoletin and scopolin are manufactured via route B. In this case, the involvement of caffeic and ferulic acids as intermediates in coumarin synthesis, as in route B, might be attributed to the abnormal conditions of tissue culture growth except that the same pathway has been detected in tobacco leaves. Although route B is thus established, it may not be widely used, since there is evidence from other tracer studies that the conversion of *p*-coumaric to umbelliferone (route A) has a central place in the biosynthesis of most plant coumarins.

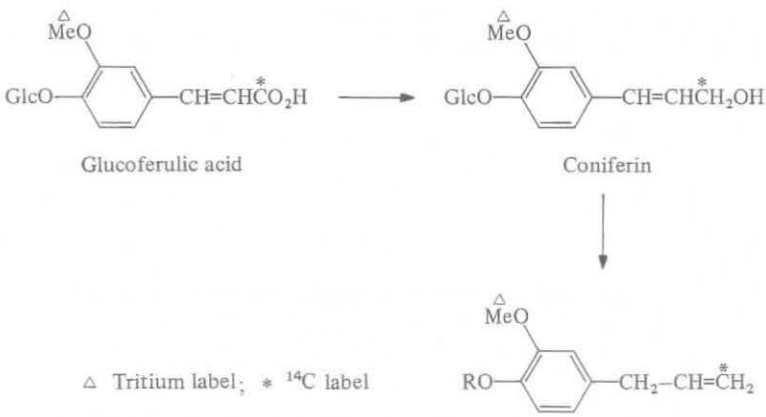


Fig. 5. Biosynthesis of eugenol and methyleugenol in *Ocimum*

4.1.4 Allyl and Propenyl Phenols

Studies of the biosynthesis of allyl and propenylphenols in plants have given rise to some controversy. Labelling experiments in basil (*Ocimum basilicum*) suggested to CANONONICA et al. (1971) that the allylphenol, eugenol, was formed from ferulic acid by loss of a terminal sidechain carbon and its subsequent replacement by a C_1 unit derived from methionine. Loss of sidechain carbon did not occur, however, in the biosynthesis of the isomeric propenylphenol, isoeugenol. A pathway involving loss of C_1 seems improbable and indeed more critical feeding experiments in the same plant, using labelled ferulic acid instead of labelled phenylalanine as precursor, have shown that both eugenol and methyleugenol are formed from ferulic acid by the more likely route without loss of carbon (Fig. 5) (KLISCHIES et al., 1975).

These differing results indicate some of the problems of interpretation in plant-feeding experiments and in particular the possibility that fed precursors, especially those remote from the end product (e.g., phenylalanine), may undergo "abnormal" metabolism instead of being incorporated into a desired biosynthetic route.

4.1.5 Flavonoids

The biosynthetic origin of flavonoids was established in feeding experiments carried out by NEISH and others with buckwheat and red cabbage in the early 1950's, and has been extensively studied since then, largely by GRISEBACH and his co-workers. A vast body of information is thus available now (for summary see HAHLBROCK and GRISEBACH, 1975) so that the main outline of the pathway and the inter-relationships of the different classes of flavonoids are very clear (Fig. 3). The only major change in the sequence of events in synthesis leading from phenylalanine to the more highly oxidised end-products of synthesis is that it is no longer necessary to include chalcones as intermediates in the main pathway. Thus, recent enzymic studies establish that the flavanone naringenin is the primary product of the key condensation of *p*-hydroxycinnamyl CoA with three malonate

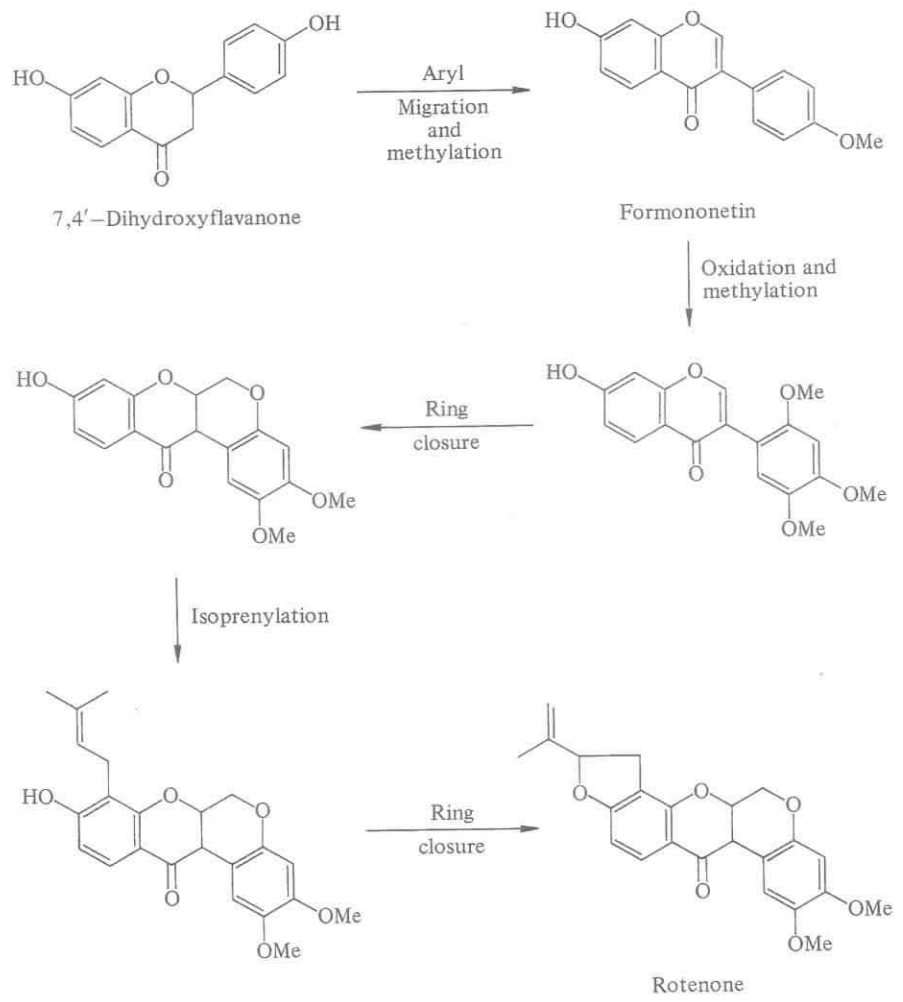
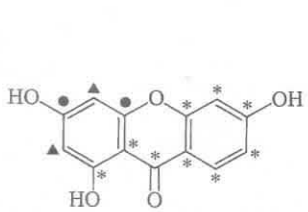


Fig. 6. The biosynthesis of the isoflavonoid rotenone

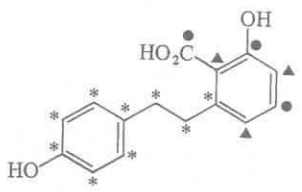
units (see Sect. 4.2) so that the obligatory intermediacy of 2′,4′,6′,4-tetrahydroxychalcone has been eliminated.

The precise mechanism of conversion of dihydroflavonol to anthocyanidin (e.g., dihydroquercetin → cyanidin, Fig. 3) has yet to be determined, but progress in studying anthocyanin biosynthesis has been aided by the realisation that most of the reactions take place partly or completely on membranes. There is still some doubt about the stage at which the second and third hydroxyl groups are introduced into the B-ring, but most evidence points to such hydroxylation occurring at the dihydroflavonol stage to give quercetin and myricetin respectively. Isoflavonoids are formed by a special aryl migration occurring very early on in synthesis (see Fig. 3).

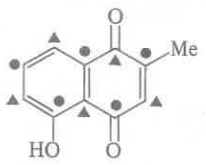
The detailed pathway to an isoflavonoid such as rotenone is now known, the various steps in the synthesis having been established by feeding experiments in young seedlings of *Amorpha fruticosa* (CROMBIE et al., 1971; Fig. 6).



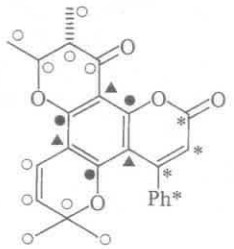
Xanthone: gentisein
Gentiana lutea
(GUPTA and LEWIS 1971)



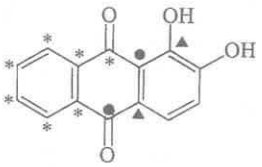
Stilbene: lunularic acid
Lunularia cruciata
(PRYCE 1972a)



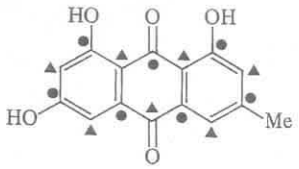
Naphthoquinone: plumbagin
Plumbago europeaus
(DURAND and ZENK 1971)



4–Phenylcoumarin: inophyllide
Calophyllum inophyllum
(GAUTIER et al. 1972)



Anthraquinone: alizarin
Rubia tinctorum
(LEISTNER and ZENK 1968)



Anthraquinone: emodin
Rhamnus frangula
(LEISTNER 1971)

▲ Methyl carbon/acetate; ● Carboxyl carbon/acetate
* Shikimate carbons; ○ Isoprenyl carbons

Fig. 7. Biosynthetic origin of carbon skeletons of various quinones and other phenolics

4.1.6 Other Phenolics

The carbon pathways of many other groups of plant polyphenols have been elucidated by feeding experiments and some typical results are shown in Figure 7. In the case of stilbenes and xanthones, very few experiments have been carried out to date so that our present knowledge of the various steps along the pathway is distinctly limited. It is possible that for a given class of compound more than

one pathway may operate. Indeed, there is evidence for two routes to xanthones, since feeding experiments on mangiferin biosynthesis in *Anemarrhena asphodeloides* indicate it is formed by a different route from that indicated for gentisein in Figure 7, one involving the intact incorporation of *p*-coumaryl CoA (FUJITA and INOUE, 1977).

In the case of the quinones, there are considerable problems in predicting the biosynthesis of any particular pigment because there are at least five known pathways to benzoquinones, five to naphthoquinones, and two to anthraquinones. One of the simplest routes to naphthoquinones is by polyketide synthesis via acetate and malonate, a pathway commonly followed in microorganisms. As indicated in Figure 7, plumbagin is formed in *Plumbago* roots in this way. Other closely similar naphthoquinones, e.g., lawsone (2-hydroxynaphthoquinone) from *Impatiens balsamina*, are, however, shikimate-derived. Only in the case of anthraquinones is the choice of pathway more limited. Typically, alizarin is formed in *Rubia* from shikimate, acetate, and mevalonate, while emodin is formed in *Rhamnus* purely from acetate (malonate) (see Fig. 7). Further details of the pathways to quinones are given in the recent comprehensive review of BENTLEY (1975).

4.2 Enzymology

4.2.1 Introduction

Spectacular progress has been made in advancing our knowledge of phenolic biosynthesis through enzymic investigations; this is particularly true in the case of phenylpropanoids, flavonoids, and lignin. The results of studying the specificities of the enzymes that catalyse the synthesis of phenolic compounds have been especially important in confirming and extending our knowledge of the intermediates in synthesis. There have been occasional conflicts in the results derived from tracer and enzymic studies. As already mentioned, the enzymic approach to flavonoid biosynthesis has ruled out the need to postulate chalcones as obligate intermediates.

In this section, a brief account will be given of the enzymology of biosynthesis, as far as it has been explored at the present time. Enzymic endeavours have concentrated on phenylpropanoid and flavonoid synthesis and almost nothing is known of enzymes concerned in xanthone or stilbene biosynthesis. The only other area of active investigation seems to be that of furocoumarin formation (see e.g., DHILLON and BROWN, 1976).

4.2.2 Phenylalanine Ammonia Lyase

Phenylalanine ammonia lyase (PAL), the key enzyme of phenolic biosynthesis, was first reported in *Hordeum vulgare* by KOUKOL and CONN (1961). It catalyzes the deamination of phenylalanine and by antiperiplanar elimination of the pro-*S*-proton from carbon 3 and of NH_3 from carbon 2 it produces trans-cinnamic acid (Fig. 8). Since 1961, its properties have been extensively studied and it has been detected in a very wide range of higher plants and also in many microorganisms (CAMM and TOWERS, 1977). The enzyme PAL has an average molecular weight of 330,000, a pH optimum of 8.8 and no cofactor requirements. Some

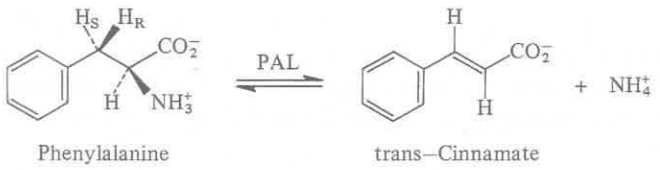


Fig. 8. The reaction catalyzed by PAL

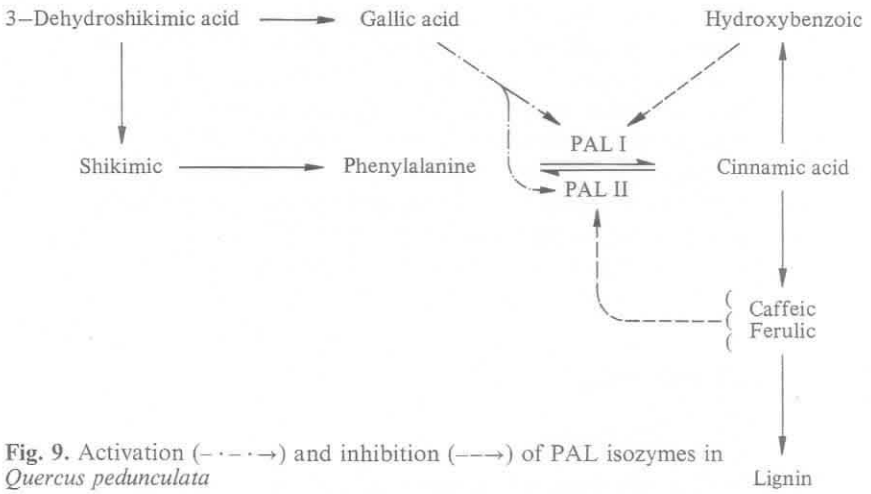


Fig. 9. Activation (– · – · →) and inhibition (——→) of PAL isozymes in *Quercus pedunculata*

preparations of PAL, especially those from grasses, show activity towards tyrosine, but it has not yet been established whether in such cases there exists a separate enzyme for the deamination of tyrosine. In general, PAL is otherwise highly specific towards its named substrate.

Most PAL preparations are inhibited by cinnamic acid and also by later products of the phenolic pathway (e.g., *p*-coumarate, kaempferol, etc.). BOUDET et al. (1971) have isolated two isozymes of PAL from *Quercus pedunculatus* leaves, which are differentially inhibited by phenolic products (Fig. 9). On the basis of these inhibition studies and of their separate compartmentation within the cell, BOUDET et al. suggest that PAL I is required for benzoic acid synthesis, while PAL II is specific for the lignin pathway (see Fig. 9).

Much controversy has centred on the question of how far PAL activity controls the flow of phenolic biosynthesis in plants. Evidence in favour of control through PAL is its rapid activation by light, through phytochrome, and its deactivation in the dark by complexing with a partly characterized high molecular weight inhibitor (see SMITH et al., 1977). If PAL is the control, it is, however, difficult to understand why its activities in plant tissues are generally much greater than those required for normal phenolic synthesis, and also why there is often a lack of correlation between changes in PAL levels and the rate of accumulation of phenylpropanoids in those tissues.

MARGNA (1977) has cogently argued a case that the major control on phenolic biosynthesis is through substrate supply. Thus, under critical conditions of rapid plant growth, phenylalanine will be preferentially incorporated into protein syn-

thesis *before* it becomes available in quantity for incorporation into flavonoids and other phenolics via PAL. Presumably, under conditions when protein synthesis is minimal, more phenylalanine becomes available for conversion into phenolics. This suggestion of MARGNA, while very plausible, does of course only push the point of control one further step back along the pathway. It is still necessary to define the mechanism by which phenylalanine is preferentially channelled into protein and how the substrate is also allowed to flow along the pathway into phenolic production.

4.2.3 Hydroxylases and Phenolases

The enzymic conversion of cinnamic acid to 4-hydroxycinnamic acid was first reported by NAIR and VINING (1965) in extracts from spinach leaves and the enzyme was more fully characterised by RUSSELL and CONN (1967) from *Pisum sativum*. Cinnamate 4-hydroxylase is a mixed function oxidase, which requires molecular oxygen, NADPH and mercaptoethanol; there is good evidence that cytochrome P-450 is required in vivo for this oxidation to take place. The mechanism of oxidation (Fig. 10) involves the well-known NIH shift; the H atom at position 4 in the benzene ring, which is replaced by OH, is not lost but ends up attached to carbon 3.

The enzymic oxidation of cinnamic acid in the *o*-position to give *o*-coumaric acid (Fig. 10) is a key step in coumarin biosynthesis. The enzyme has only been partly characterised because it is membrane-bound. GESTETNER and CONN (1974) have obtained it from chloroplast preparations of *Melilotus alba* seedlings and record its optimum pH as being 7.0.

The introduction of a second hydroxyl group into *p*-coumaric to give caffeic (Fig. 10) is catalysed by a well-known group of plant enzymes – the phenolases or polyphenoloxidases. These enzymes normally oxidise the caffeic acid further to the corresponding *o*-quinone and this then polymerises to dark products. The

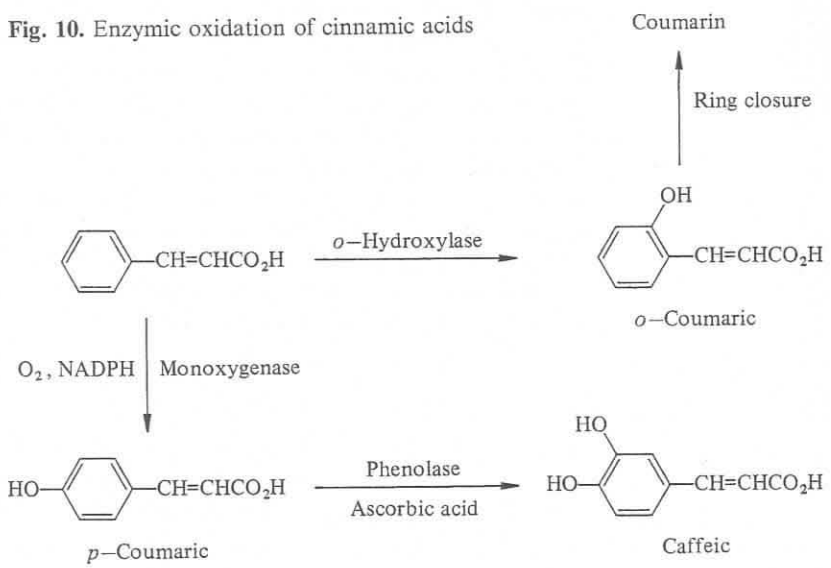


Fig. 10. Enzymic oxidation of cinnamic acids

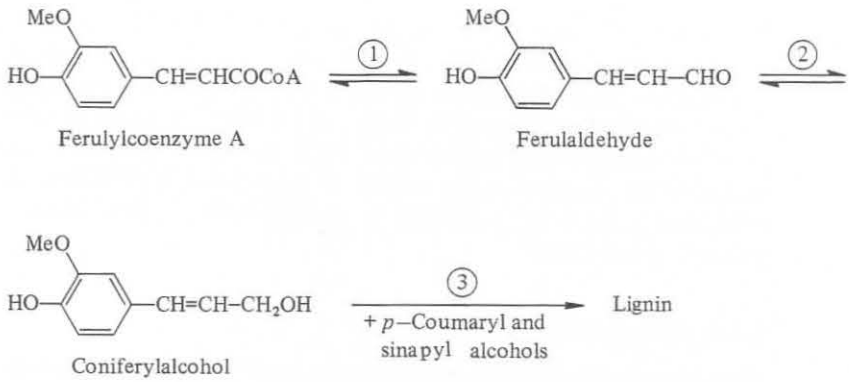


Fig. 11. Enzymes of lignin biosynthesis. *1* cinnamyl CoA NADPH reductase, *2* cinnamyl alcohol NADP oxidoreductase, *3* peroxidase

synthetic role of phenolases has been explored and, in the presence of ascorbic acid as reductant, the reaction can be limited to the first stage. Indeed, phenolase preparations from spinach beet (VAUGHAN et el., 1969) and parsley suspension cultures (SCHILL and GRISEBACH, 1973) will readily catalyse the conversion of *p*-coumaric to caffeic acid. Unlike the parsley enzyme, that from spinach beet also catalyses oxidation of naringenin, dihydrokaempferol and kaempferol in the 3′-position, so that it could be also important in flavonoid biosynthesis.

4.2.4 *O*-Methyltransferases

Enzymes capable of specifically catalysing the *O*-methylation of caffeic acid in the 3-position to give ferulic acid (3-methoxy-4-hydroxycinnamic acid) have been described from a variety of plant tissues. Like other *O*-methyltransferases, they require *S*-adenosylmethionine as methyl donor. Many of the enzyme preparations described will act on several catechol substrates, besides caffeic, but a few are more highly specific. Thus parsley suspension cultures have yielded an *O*-methyltransferase which is only really active with flavone substrates and will methylate luteolin to chrysoeriol (luteolin 3′-methyl ether) in good yield (EBEL et al., 1972). More recently, two methyltransferases have been separated from soya bean cultures, one acting on caffeic acid and being involved in lignification, the other acting only on flavonoid substrates (POULTON et al., 1976). These two enzymes are not isozymes, since they have clearly distinct substrate requirements, and presumably they have different roles in overall phenolic metabolism. Many other methylations of hydroxyl substituents occur in phenolic and particularly in flavonoid biosynthesis but the enzymes concerned have yet to be characterised.

4.2.5 Enzymes of Lignin Biosynthesis

The two enzymes required for the reduction of hydroxycinnamic acids, as their CoA esters, to the corresponding alcohols (Fig. 11) have been characterised and detected in a wide variety of plant tissues. The NADPH-dependent cinnamyl alcohol oxidoreductase has been purified 600-fold from *Forsythia suspensa* tissues and

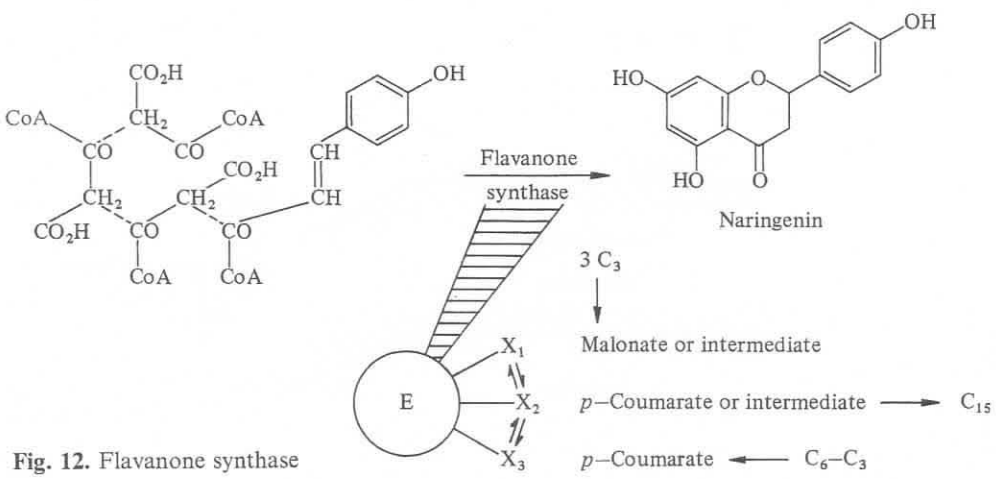


Fig. 12. Flavanone synthase

been extensively studied (MANSELL et al., 1974). In its properties, it resembles other plant alcohol dehydrogenases but is absolutely specific towards cinnamyl substrates so that its role in lignin biosynthesis is assured. It has a MW of 80,000 and a pH optimum of 7.6. In keeping with its biosynthetic importance, measurements have shown that there is ten times more activity in gymnosperm cambial tissues than in herbaceous angiosperm non-lignifying tissues.

The last stage in lignin biosynthesis is assumed to involve peroxidase, cellulose and mixtures of hydroxycinnamyl alcohols (see e.g., NAKAMURA et al., 1974). Gymnosperm lignin is based on *p*-coumaryl and ferulyl alcohols, while angiosperm lignin has sinapyl alcohol units as well. Efforts to determine the critical biochemical factors controlling these differences in lignin make up have not so far been completely successful, but the enzymes of hydroxylation and *O*-methylation of *p*-coumarate and ferulate may be most important.

4.2.6 Enzymes of Flavonoid Synthesis

Flavanone synthase which catalyses the condensation of *p*-coumaryl CoA with three malonate units to give naringenin (Fig. 12) has been isolated from parsley and *Machaeranthera* cell cultures and from leaves, flowers, and anthers of tulip (HRAZDINA et al., 1976, 1978; SALEH et al., 1978). It is highly specific for *p*-coumaryl CoA with a pH optimum of about 8.0 and is inactive with cinnamyl, *p*-methoxycinnamyl, and isoferulyl CoA esters. It is, however, very active with caffeyl CoA, giving eriodictyol, but this reaction has a different pH optimum, between 6.5 and 7.0. The fact that flavanones are formed directly in this reaction rules out earlier suggestions that chalcones were the first C_{15} compounds to be formed in synthesis. It also means that flavanone-chalcone isomerases, which are actually widespread in plants, do not have, as was supposed, a key biosynthetic role.

Flavanone synthase probably has three binding sites, as indicated in Figure 12, which catalyse the stepwise addition of malonate units to the activated *p*-coumaric or caffeic acid. Thus *p*-coumaryl CoA is first attached at X_3 and is shuttled to and fro from sites X_2 and X_1, receiving at X_1 three successive malonate units. The completed C_{15} molecule is then lost from site X_2.

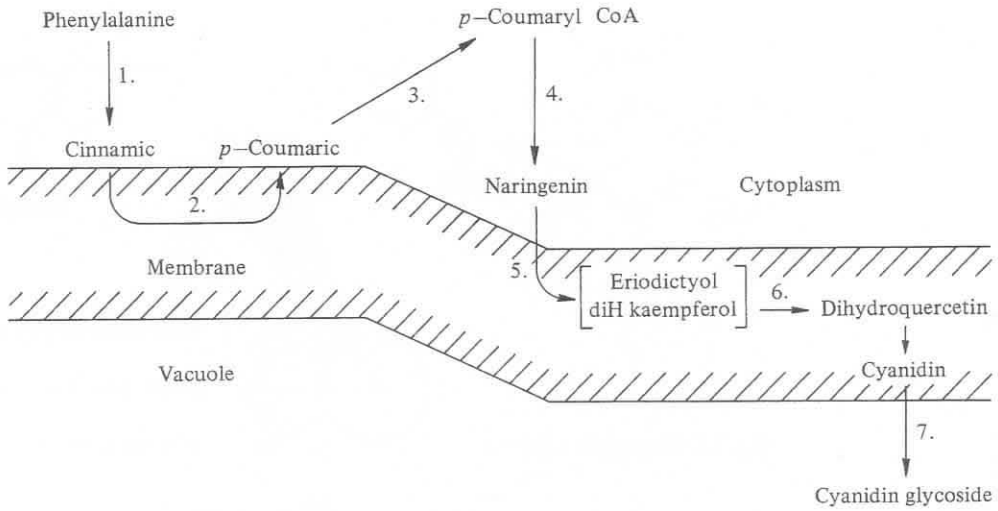


Fig. 13. Enzymology of anthocyanin biosynthesis. *1* PAL, *2* cinnamate 4-hydroxylase, *3* *p*-coumarate CoA ligase, *4* flavanone synthase, *5,6* oxidases, *7* UDP glucose:cyanidin 3-*O*-glucosyltransferase

The final step in the synthesis of flavones requires the oxidation of flavanones in the 2,3-position and a flavanone oxidase with just these properties had been detected in young primary leaves of parsley (SUTTER et al., 1975). It catalyses the oxidation of naringenin to apigenin and also of 7,4′-dihydroxyflavanone to 7,4′-dihydroxyflavone. It requires O_2, Fe^{2+} and a heat-stable, dialysable cofactor for its operation. Notably, it fails to oxidize chalcones to flavones and this again shows (see above) that flavanones and not chalcones are key intermediates in flavonoid synthesis.

The biosynthesis of anthocyanins has been examined enzymically (FRITSCH and GRISEBACH, 1975) especially in suspension cultures of *Machaeranthera*, and the pathway is probably as shown in Figure 13. Unfortunately, the enzyme catalyzing the step: dihydroquercetin → cyanidin has so far eluded detection. However, a major step forward in comprehending pigment synthesis has been the realisation that most of the enzymic reactions take place partly or completely on membrane surfaces. Recent experiments with leaves and petals of *Hippeastrum* and *Tulipa* on the subcellular localisation of these enzymes suggest that their highest activity is in the cytosol fraction of the protoplasts (HRAZDINA et al., 1978).

4.2.7 *O*-Glycosyltransferases

Enzymes capable of transferring glucose from UDP glucose to phenolic hydroxyl groups, as in hydroquinone, have been known for a long time and the enzymic synthesis of hydroxycinnamic acid glucose esters in the presence of UDP glucose has also been well described. Similar glycosyl transferases are also involved in the final stages of flavonoid synthesis and such catalytic activities have now been detected in over a dozen plants (Table 4). The four main glycosyl transferases detected mainly operate on only one class of flavonoid and they catalyse the

Table 4. Source of glycosyl transferase enzymes in flavonoid synthesis

UDP Glucose: quercetin 3-*O*-glucosyltransferase	
Brassica oleracea seedlings	*Phaseolus aureus* leaves
Impatiens balsamina petals	*Ph. vulgaris* leaves[a]
Leucaena glauca leaves	*Zea mays* pollen
Petroselinum crispum cells	
TDP Rhamnose: quercetin 3-*O*-glucoside *O*-rhamnosyltransferase	
Phaseolus aureus leaves	*Ph. vulgaris* leaves
UDP Glucose: cyanidin 3-*O*-glucosyltransferase	
Brassica oleracea seedlings[b]	*Machaeranthera* cells
Hippeastrum leaves	*Tulipa* leaves, petals
UDP Glucose: apigenin 7-*O*-glucosyltransferase[c]	
Petroselinum crispum cells	

[a] Also contains a glucuronic acid transferase enzyme
[b] Enzyme from this source will also glucosylate flavonols and other anthocyanidins, besides cyanidin
[c] This enzyme will also glucosylate flavonols in the 7-position; a second enzyme from parsley cells has been characterized which will transfer apiose to apigenin 7-glucoside to give the 7-apiosylglucoside (see HAHLBROCK and GRISEBACH, 1975)

addition of sugar specifically to a particular hydroxyl (either in the 3- or 7-positions). The glycosyl donor molecule is normally UDP glucose for glucosylation and TDP rhamnose for rhamnosylation, but at least in vitro the enzymes will transfer sugars from other donor molecules (see HAHLBROCK and GRISEBACH, 1975).

Occasionally, the sugars of flavonoid glycosides carry acyl components and the enzymology of such acylation has been explored in at least two cases. Thus, a malonyl transferase adding malonic acid to apigenin 7-apiosylglucoside has been described in parsley cultures (HAHLBROCK and GRISEBACH, 1975). A similar enzyme catalysing the transfer of *p*-coumaric acid to kaempferol 3-triglucoside has been reported in leaves of *Pisum sativum* (SAYLOR and MANSELL, 1977).

4.3 Physiology of Biosynthesis

Physiological aspects of flavonoid formation in plants were extensively reviewed by BLANK in Volume X of the Encyclopedia of Plant Physiology, first series. More recent work has been critically reviewed by MCCLURE (1975) and excellent accounts are available on the photocontrol of flavonoid biosynthesis via phytochrome (SMITH, 1972) and of the regulation of PAL synthesis in plants (SMITH et al., 1977). Less has been written about the physiology of formation of other classes of phenol, but presumably many of the parameters affecting flavonoid production apply to phenolic compounds in general, since they are almost all formed ultimately via the key enzyme system PAL. The present position regarding the major physiological variables in flavonoid synthesis is summarised in Table 5.

A major problem in interpreting the results of physiological studies in this area is that rarely, until recently, has the concentration of more than one type

Table 5. Physiological factors in phenolic synthesis

Light
1. low energy R⇌FR phytochrome system general requirement in all plants studied.
2. High Intensity Response (HIR), either FR or blue light often a requirement for anthocyanin synthesis.
3. Photosynthetic involvement for precursor or cofactor a presumed requirement for anthocyanin synthesis in tuber or root.
4. UV light occasionally induces synthesis or may augment it.
5. Ionizing radiation increases anthocyanin levels.
Temperature
Lower temps. generally favour increased anthocyanin and isoflavone levels in leaves. Exceptionally, proanthocyanidin synthesis is depressed by lowering temp.
Water
Waterlogged plants may show increased flavonoid production, while desiccated plants (20% water deficit) in general produce less phenolic
Carbohydrate
General, sometimes marked, increase in synthesis.
Growth Hormones
Response unpredictable, depending more on other factors.
Mineral Nutrition
N or P deficiency usually increase flavonoid levels. B deficiency may increase hydroxycinnamic acid levels at the expense of flavonoids or lignin.

of flavonoid constituent been determined. In fact, attention has nearly always focused on anthocyanin, since it is so easy to measure it by spectrophotometry at 520 nm. Unfortunately, in determining the effect of any physiological variable on phenolic synthesis, it is necessary to examine all major classes, since they are all formed on a biosynthetic metabolic grid. An inhibition in one pathway may be correlated with an increase in synthesis along another. It is possible, for example, for some physiological factor to inhibit anthocyanin synthesis by perhaps 50%, but at the same time to increase flavonol glycoside synthesis by, perhaps, 300%. If levels of both classes are approximately the same in the control, then the change in the physiological parameter in reality has on overall beneficial effect on flavonoid production, even though if anthocyanin levels alone are measured it would appear to be harmful to synthesis.

Where the effect of light has been studied on more than one end product of synthesis, it has been found to have different effects on different pigments and the effects produced depend on the tissue used. For example, in the case of *Spirodela intermedia*, dark-grown plants only synthesise flavonoids with a monohydroxylated B-ring, i.e., vitexin and kaempferol 3-glucoside. Plants grown under low intensity white light start synthesising compounds with a dihydroxylated B-ring, namely orientin and quercetin 3-glucoside. Only high light intensity for several

days will induce anthocyanin (cyanidin 3-glucoside) synthesis in addition (McClure, 1968). Again, with flavonol glycoside synthesis in *Pisum sativum* (Galston, 1969), brief red light causes the accumulation of quercetin glycoside in the plumule and kaempferol glycoside in the internode tissues, whereas both groups of flavonol glycoside are synthesised in the leaf.

Many observations have been made on the effect of adding organic precursors, plant hormones, enzyme inhibitors and inorganic salts on phenolic synthesis in plants. Rarely, however, do any clear-cut conclusions emerge from such studies (see Table 5). One common observation, viz. that adding sugar nearly always stimulates phenolic synthesis more than adding a known precursor, continues to pose a problem in terms of the accepted routes of biosynthesis. Creasy and Swain (1966) in their study of phenolic synthesis in strawberry leaf discs, however, found that high sugar concentrations could actually mask the usual phytochrome response in flavonoid synthesis. Clearly, in such cases, the results of artificial feeding experiments have to be interpreted with caution.

4.4 Turnover and Metabolism

It is now widely accepted by plant biochemists that phenolics are not end products that accumulate unchanged in plant cells. Instead, they are thought to be part of a dynamic equilibrium in which, even when produced in quantity, there is continual synthesis, turnover, and degradation. Turnover can be particularly rapid in floral tissues, since in many angiosperms, the requirement for floral pigmentation is quite short, i.e., only a day or a few days while the floral organs are ripe for pollination. This is true in *Petunia hybrida*, where the half life of anthocyanins based on delphinidin has been determined by tracer studies to be between 25 and 31 h (Steiner, 1971).

A study of anthocyanin synthesis in relationship to flower development in the pea flower has also demonstrated the presence of a very active system of synthesis and turnover. As the pea flower senesces, the supply of "synthetic" enzymes runs out and the pigments produced lack some of the methyl and sugar residues that are present in the pigments of the flower at maturity (Statham and Crowden, 1974). Similar studies in *Impatiens* (Hagen, 1966) and other flowers have shown a progressive change with time in pigment patterns. The catabolism of these floral anthocyanins is not fully understood, but one of the first steps is the removal of sugar from the 3-hydroxyl and specific glycosidases, called anthocyanases, are known which carry out this step. Another catabolic reaction in the case of anthocyanidins with catechol nuclei (e.g., cyanidin) is oxidation and phenolase or peroxidase-type enzymes have been reported in plant tissues (e.g., cherry fruit) where cyanidin glycosides are decolorised.

Leaf flavonoids are probably turned over more slowly than floral pigments, but there is good evidence that metabolism occurs. Pulse-labelling measurements on kaempferol and quercetin glycosides in *Cicer arietinum* leaves have clearly indicated a half life of 7 to 12 days (Barz and Hösel, 1971). The catabolism of flavonols involves at an early stage the addition of oxygen to the 2,3-double bond to give a 2-hydroxy derivative (Fig. 14). This 2,3-dihydroxyflavanone is then ring-cleaved to give a substituted benzoic acid and a phloroglucinol derivative,

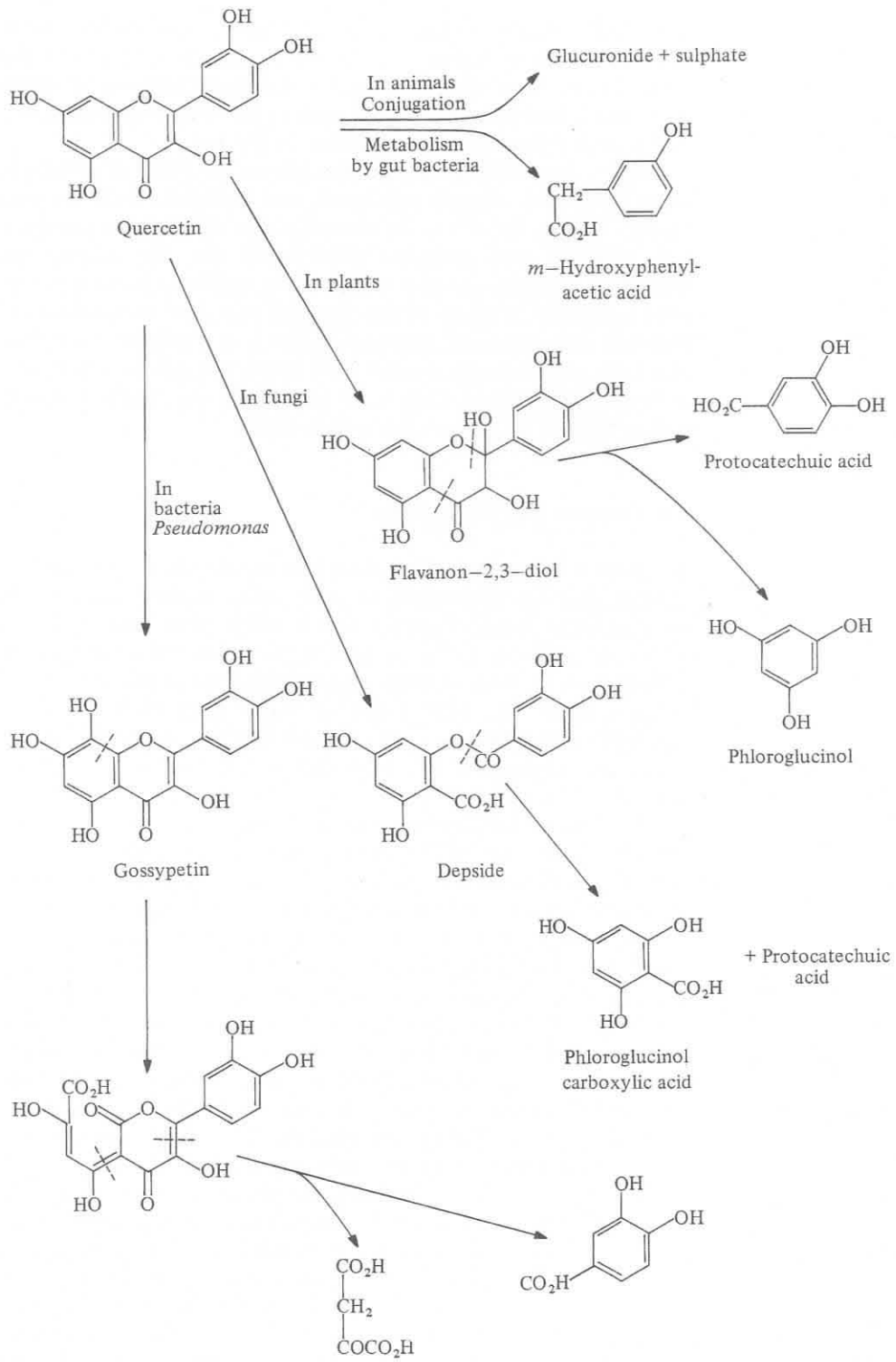
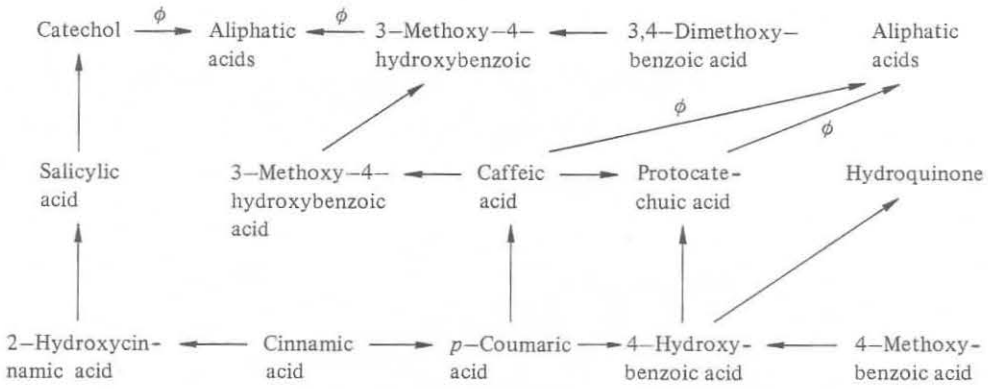


Fig. 14. Metabolic fate of the flavonol quercetin in animals, plants, and micro-organisms



ϕ = Ring cleavage

Fig. 15. Metabolic fate of simple phenols in plants

which may not be detectable because it so readily undergoes further oxidation. Undoubtedly the end products are eventually returned to the atmosphere as respired CO_2.

The catabolism of other leaf flavonoids has been less well studied, but it appears that flavanones may be oxidised by ring cleavage at the 2-position to furnish 5,7-dihydroxychromones and hydroxybenzenes (see Sect. 2.2), which are then further broken down in the same way to give CO_2 as their final fate. Alternatively, flavanones may undergo isomerisation to chalcones, which are then metabolised by the pathway: hydroxycinnamic acid $\rightarrow \rightarrow$ hydroxybenzoic acid $\rightarrow \rightarrow \rightarrow CO_2$.

The fate of simpler phenolic compounds in plant tissues has been extensively studied (ELLIS, 1974) and is summarised in part in Figure 15. One main reaction is the β-oxidation of hydroxycinnamic acids to hydroxybenzoic acids. Also methoxybenzoic acids may undergo demethylation and/or decarboxylation. In general, catechol derivatives are formed and these finally undergo ring cleavage to aliphatic acids and then to CO_2. Recent experiments have shown that higher plants possess active aromatic ring-cleaving enzyme systems, similar to those well known in microorganisms, and ring fission is a common feature of phenolic turnover in plants. Ring-cleaving enzymes have also been detected in plant tissue cultures and the catabolism of phenolics in cell cultures has been well studied (BARZ and HÖSEL, 1975; BARZ, 1977).

From the viewpoint of comparative biochemistry, it is instructive to relate the fate of a flavonol such as quercetin in plant tissues with its fate in animals and microbes (Fig. 14). In animals the major reaction that phenolics undergo on feeding is conjugation, especially with glucuronic acid but also with ethereal sulphate (WILLIAMS, 1964). Considerable degradation may also occur in vivo but this is essentially due to the activities of enzymes produced by the gut bacteria (GRIFFITHS and BARROW, 1972). Dehydroxylation of aromatic compounds is a special feature of such bacterial degradation and quercetin, on feeding to a range of animals, is often recovered as *m*-hydroxyphenylacetic acid. By contrast, in the fungal turnover of quercetin, the first reaction is cleavage at the heterocyclic ring by quercetinase, with the expulsion of the 3-carbon atom as carbon monoxide – the resulting depside (Fig. 14) then undergoes esterase attack with the production

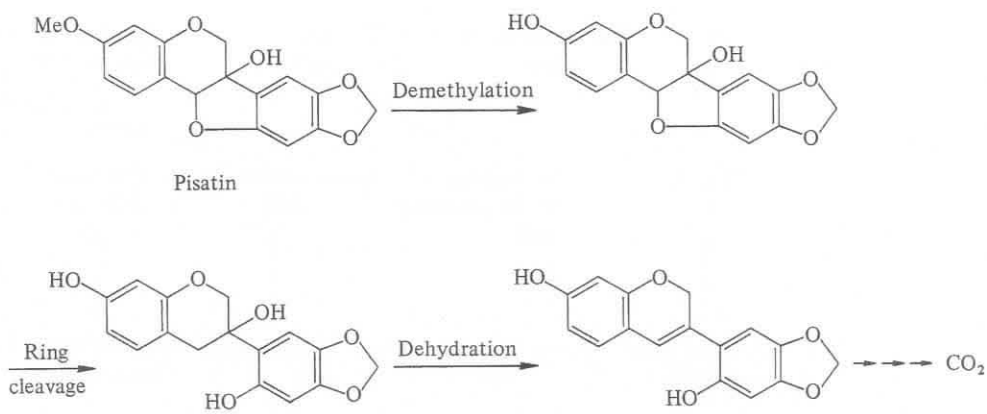


Fig. 16. Metabolism of the pterocarpan pisatin by the fungus *Fusarium oxysporum*

of phloroglucinol carboxylic and protocatechuic acids. Bacteria are also capable of catabolizing quercetin and employ yet another degradative route. Thus, in *Pseudomonas*, the first step is the introduction of oxygen at the 8-position to give gossypetin (8-hydroxyquercetin). This then undergoes specific ring cleavage at the C-C bond adjacent to the introduced oxygen, with the eventual production of protocatechuic and aliphatic acids (Fig. 14).

Less is known of the comparative catabolism of isoflavonoids in different organisms, but the in vivo reduction in mammals of isoflavones to isoflavans has been actively studied, because the isoflavans produced have oestrogenic effects in farm animals (see SHUTT, 1976). The fungal modification of isoflavonoids has also been of particular interest, because of the role of certain of these phenolics as antifungal agents produced in legume plants de novo in response to fungal infection (see HARBORNE and INGHAM, 1978). Fungal metabolism includes introduction of hydroxyl groups and demethylation of methoxyl substituents; the metabolites are usually significantly less fungitoxic than the original isoflavonoids. Only the first products of metabolism have been characterised. Such metabolites then apparently undergo ring-cleavage reactions and the small molecules so produced are rapidly dissimilated. One of the best known isoflavonoid phytoalexins is pisatin from *Pisum sativum* and its metabolism by the pea pathogen *Fusarium oxysporum* is illustrated in Figure 16.

5 Phenolic Production in Tissue and Cell Culture

5.1 Introduction

Phenolic synthesis is a characteristic and universal feature of plant metabolism and it is a property which extends to plants grown in organ culture, as undifferentiated callus tissue or in cell suspension culture. While the pattern of products

may be dramatically altered under tissue culture conditions, nevertheless the same phenolic materials continue to be made in such cells. Initially, the phenolic concentrations may be very low and the compounds produced of the simplest type; on subculturing, however, strains may be obtained with enhanced synthetic ability. In general, the substances formed in tissue culture are the same as those in the intact plant and as indicated in Table 6, almost every class of phenolic encountered in plants can be found in tissue culture as well. Unfortunately, the phenolic repertoire of a cell culture has rarely been compared in detail with that of the corresponding intact plant, so that it is still not certain whether the few differences that have been noted in culture are real or apparent.

Tissue culture studies have been most important in providing information on the enzymology of phenolic biosynthesis. Indeed our present sound basis of knowledge of the pathways of flavonoid production (see Sect. 4.1) could not have been obtained without the extensive use of parsley and soya bean tissue cultures as convenient sources of enzyme activities. In many cases, extraction and characterisation of new enzymes along the pathway using tissue-cultured cells has been an essential preliminary to the often more daunting task of detecting the same activity in whole plants.

Several accounts of phenolic production in plant tissues can be found inter alia in general reviews such as those of BARZ et al. (1977), BUTCHER (1977) and STREET (1977). Here, attention will be focused on the synthetic capacity of tissue-cultured cells and on the regulation of such synthesis.

5.2 Qualitative Aspects

The fact that anthocyanins are produced in tissue culture was realised over two decades ago, when STEWARD (1958), one of the early pioneers of the technique, recorded that some callus tissues derived from carrot *Daucus carota* turned deep red following subculturing. Since then, a range of plants has been found which consistently produces anthocyanin pigments in callus or shake culture. One of the most striking examples is *Machaeranthera gracilis*, which produces relatively large amounts (usually 3% dry wt.) of the 3-glucoside and 3-rutinoside of cyanidin in cultured cells. It is remarkable in that the plant from which these cultures are derived shows no signs of anthocyanin colour in normal growth. However, the two cyanidin derivatives formed in culture are those characteristic of plants of the Compositae, to which family *Machaeranthera* belongs (see HARBORNE, 1978a), and no doubt this plant could be induced to produce the same pigments in leaves or stems by subjecting it to some kind of physiological stress. In fact, in almost all cases where a proper comparison has been made of anthocyanin pigments in culture and the whole plant (e.g., in *Dimorphotheca* and in *Solanum tuberosum*, see Table 6) the same pigments have been found. Thus, the capacity for anthocyanin synthesis normally reflects that found in the intact tissue from which the callus has been derived.

One possible anomaly is flax tissue culture, which is reported to be coloured by cyanidin 3,5-diglucoside (see Table 6). In this case, the pigments have been fully analysed in flowers and leaves of various flax genotypes (DUBOIS and HARBORNE, 1975) and although cyanidin is one of the aglycones present, it occurs

Table 6. Phenolics identified in plant callus or suspension cultures

Plant	Substances identified	References
Anthocyanins		
Daucus carota (carrot)	Cyanidin 3-glycosides[a]	SCHMITZ and SEITZ (1972)
Dimorphotheca sinuata	Cyanidin and delphinidin 3-glucosides	BALL et al. (1972)
Linum usitatissimum (flax)	Cyanidin 3,5-diglucoside (?)	IBRAHIM et al. (1971)
Machaeranthera gracilis[b]	Cyanidin 3-glucoside and 3-rutinoside (also quercetin glycosides and chlorogenic acids)	HARBORNE (1964b); STICKLAND and SUNDERLAND (1972)
Parthenocissus quinquefolia	Cyanidin 3,5-diglucoside (and quercetin 3-glycosides)	BLEICHERT and IBRAHIM (1974)
Solanum tuberosum (potato: cv. Congo)	Negretein (acylated malvidin 3-rutinoside-5-glucoside)	HARBORNE and SIMMONDS (1962)
Other flavonoids		
Camellia sinensis (tea)	Quercetin 3-rutinoside, catechins and procyanidins	FORREST (1969)
Cicer arietinum (chickpea)	Formononetin and biochanin A	BARZ (1977)
Citrus cvs.	Sinensetin, nobiletin and other methylated flavones	BRUNET and IBRAHIM (1973)
Glycine max (soya bean)	Apigenin and other flavones	BARZ (1977)
Petroselinum crispum[c] (parsley)	Apigenin 7-apiosylglucoside and 23 other flavones	KREUZALER and HAHLBROCK (1973)
Phaseolus aureus	Daidzein, 2′4′4-trihydroxychalcone, coumestrol and soyagol	BERLIN and BARZ (1971)
Ph. vulgaris (bean)	Phaseollin	INGRAM (1977)
Pisum sativum (pea)	Pisatin	BAILEY (1970)
Silybum marianum	Silybin	SCHRALL and BECKER (1977)
Theobroma cacao (cocoa)	(−)-Epicatechin, procyanidins, flavanones (?) (*p*-coumaric and caffeic acids)	JALAL and COLLIN (1977)
Phenylpropanoids		
Ammi visnaga	Visnagin (chromone)	KAUL and STABA (1967)
Coleus blumei	Rosmarinic acid	RAZZAQUE and ELLIS (1977)
Nicotiana tabacum	Scopolin, scopoletin and hydroxycinnamic acid esters	FRITIG et al. (1970)
Ruta graveolens	Umbelliferone, bergapten, psoralen and other coumarins	STECK et al. (1971)

[a] Pigments in carrot cultures have not been fully characterised but it is likely that they are identical to those reported in the intact carrot plant: cyanidin 3-lathyroside, cyanidin 3-xylosylglucosylgalactoside and its ferulyl derivative (see HARBORNE, 1976a)

[b] This is now the correct name for the plant referred to in the literature as *Happlopappus gracilis*

[c] This is now the correct name for the plant referred to in the literature as *Petroselinum hortense*

Table 6 (continued)

Plant	Substances identified	References
Quinones		
Cassia tora	Chrysophanol, emodin, physcion	TABATA et al. (1975)
Digitalis lanata	Digitolutein and five other anthraquinones	FURUYA et al. (1972)
Lithospermum erythrorhizon	Shikonin and related naphthoquinones	TABATA (1977)
Morinda citrifolia	Alizarin, damnacanthal, morindone	ZENK et al. (1975)
Plumbago zeylanica	Plumbagin	HEBLE et al. (1974)

in petals as the 3-glucosylrutinoside, 3-triglucoside, 3-rutinoside or 3-glucoside. The report by IBRAHIM et al. (1971) of the 3,5-diglucoside in cell culture is, therefore, unexpected. However, it could well be a misidentification, since the same authors (THAKUR and IBRAHIM, 1974) spuriously reported some unusual methylated pigments in the hypocotyl of flax seedlings, which on re-examination was found to contain only cyanidin 3-rutinoside (DUBOIS and HARBORNE, 1975). It should also be noted (a point not realised by BUTCHER (1977) in his review) that the earlier report by ARDENNE (1965) of cyanidin 3,5-diglucoside in *Machaeranthera* was erroneous. The second pigment in this culture is the 3-rutinoside (HARBORNE, 1964b), an identification that has been confirmed by later workers (STICKLAND and SUNDERLAND, 1972).

A range of other flavonoids and of hydroxycinnamic acid derivatives have been identified variously in tissue cultures (Table 6) and the compounds are usually the same as those produced in the parental plants although this point has not always been checked. The most elaborate investigation has been of parsley tissue cultures, which produce 24 flavones. While 14 of these have been identified, it is not clear how many of these constituents actually occur in intact parsley plants: only a few have been recorded positively in seed or leaf tissue.

One plant where a direct comparison has been conducted between phenolic patterns in cotyledon, leaf, stem, and callus derived from these tissues is *Theobroma cacao* (JALAL and COLLIN, 1977). While there are anthocyanins and flavonol glycosides in the cotyledons and glycoflavones in the leaves, none of these substances was detected in the respective callus tissues. All four callus clones had the same pattern, containing epicatechin and two procyanidins, three reduced flavonoids uniformly present throughout the cocoa plant. Several apparently new flavanone-like derivatives were also present in callus. Whether these unidentified constituents are really distinctive of tissue culture is not clear, since they may be formed in the plant in amounts below the normal level of detection. Alternatively they may represent biosynthetic intermediates of the more highly oxidised flavonoids of intact tissue, which accumulate because one or more of the enzymes for flavonoid oxidation are absent from the culture.

Hydroxycinnamic acids have been widely observed in cultured cells, occurring both free and combined. Apparently "new" conjugates of *p*-coumaric, caffeic, and ferulic with the diamine putrescine were found for the first time in tobacco

callus by MIZUSAKI et al. (1971). Subsequent investigations, however, have shown that these conjugates are not peculiar to tissue culture, but occur in whole plants, in tobacco and in other species (TANGUY et al., 1978).

One situation where tissue cultures produce phenolics not present in healthy plants is that of phytoalexin synthesis in legumes such as *Pisum* and *Phaseolus*. The pterocarpans pisatin and phaseollin are not detectable in healthy leaves or stems of peas and beans, but are specifically produced in these tissues in response to fungal invasion. What is remarkable, therefore, in the tissue cultures of these two legumes is their ability to synthesise pterocarpans without the "trigger" mechanism necessary in intact tissue. In the case of *Pisum sativum*, the necessary stimulus is provided by the coconut milk in the medium (BAILEY, 1970). It is clear from other pathological studies in tissue culture that normal resistance mechanisms to disease may be modified by the special physiological conditions of cell culture. For example, potato tissue cultures derived from plants with R gene resistance against blight, on infection with *Phytophthora infestans* spores, produce a toxic mixture of salicylic, vanillic and *p*-hydroxybenzoic acids as a response. By contrast, in tubers with R gene resistance, the fungus induces synthesis of the sesquiterpenoid rishitin (see INGRAM, 1977).

In conclusion, then, there is at present little evidence for the synthesis of any phenolics in cell cultures which are not produced at some stage or other in the life cycle of the plant. The only suggestion of abnormal synthesis seems to be in lignification in tissue culture, since NIMZ et al. (1975) have found in soya bean cultures that there are simple cinnamyl residues in the lignin and also a specific reductase converting cinnamic acid to cinnamyl alcohol. Such residues have not so far been recorded as monomeric units in normal plant lignin. It may be noted, however, that lignification in tissue culture is somewhat aberrant generally, since whatever the plant used for producing callus, the lignin in the culture is essentially the same, based almost entirely on guaicyl residues (see BUTCHER, 1977).

5.3 Metabolic Aspects

One of the major features of plant tissue culture is that many of the normal restraints on metabolism are absent or at least may be over-ruled by hormonal or light treatment, so that theoretically it is possible to obtain clones which make massive amounts of a particular phenolic. Caffeic acid ester (e.g., chlorogenic acid) levels in plant leaves, for example, rarely exceed 1% dry wt. while caffeic acid derivatives may be formed in vast excess of this – up to 10% dry wt. – in cell cultures of *Machaeranthera* (HARBORNE, 1964b) and *Coleus* (RAZZAQUE and ELLIS, 1977). Such clones are ideal for studying the biosynthesis of these phenolic components and would also be valuable for commercial production, if these end products were of industrial value. Yields of phenolics normally vary in callus cultures and stable high-yielding strains may only be obtained after repeated selection and subculturing (MIZUKAMI et al., 1978).

The major success in producing large quantities of medicinally useful phenolics in tissue culture has, however, been with hydroxyquinones (TABATA, 1977). A number of anthraquinone derivatives are the active principles of plant drugs used pharmaceutically as laxatives and it is interesting that callus tissue of one such

plant *Cassia tora* will produce ten times the normal amount of these quinones – up to 6% dry wt. Suspension cultures of *Morinda citrifolia* are even more rewarding and yield 10% dry wt. of total anthraquinone. Finally, callus of *Lithospermum erythrorhizon* produce the record amount of 12% dry wt. naphthoquinones, compounds used medicinally in the treatment of skin diseases and burns.

One other way of utilising tissue culture to produce medicinally useful phenolics is to feed in "unnatural" precursors and obtain modified metabolites as a result. The feasibility of this approach has been demonstrated by SCHRALL and BECKER (1977) with *Silybum marianum* (Compositae) cultures. When supplied with taxifolin (dihydroquercetin) and coniferyl alcohol, these cultures produced silybin, one of the main flavonolignans of the *Silybum* plant. The same cultures supplied with luteolin instead of dihydroquercetin were able to produce hydnocarpin, a flavonolignan previously only known as a plant product in a different family, in *Hydnocarpus wightiana* (Flacourtiaceae). Medicinally, flavonolignans are of value in treating liver ailments (WAGNER, 1978) and feeding yet other flavones to these cells might conceivably yield new structures with enhanced pharmacological activities.

The ability of tissue cultures generally to take up and metabolise exogenously supplied phenolics has been discussed by BARZ (1977) in some detail. As is the case with whole plants, one of the first reactions is conjugation with glucose and phenolic acids supplied to tissue cultures are converted to the corresponding glucose esters. During feeding experiments it seems likely that such conjugation may prevent the incorporation of fed molecules into "new" biosynthetic routes. Undoubtedly, also, substances fed into cell cultures will eventually undergo turnover and degradation and many reactions of this type have been observed in such experiments. The catabolism of exogenously supplied phenolics is similar to that of endogenous substances, although sometimes polymerisation reactions are more in evidence.

Much use has been made of tissue cultures for biosynthetic studies in the assumption that the pathways followed are the same as in the plants from which the cultures have been obtained. Whether this assumption always holds true is open to question, since it has not often been deliberately tested. It does seem to be true for flavone synthesis in parsley cells and leaves, and also for anthraquinone synthesis in suspension cultures and seedlings of *Morinda citrifolia* (LEISTNER, 1973) but further comparisons could usefully be carried out in other cases.

6 Functions of Phenolics in Plants

6.1 Physiological Interactions

There is still considerable uncertainty as to whether phenolic compounds have a physiological role in plant growth and metabolism. Many phenols are clearly able to exert significant effects on growth processes when applied to plant tissues at physiological concentrations, but this does not necessarily imply they have an endogenous role. With so much variation in structure, it is unlikely that phenolics as a group of substances have one particular universal role in regulating growth

and development. Rather, it is possible that individual classes or individual substances may possess significant activities in certain physiological processes. Many of the observed physiological interactions may be incidental to the wider function of phenolics as protective agents in plants (see SWAIN, 1977). The putative physiological role of phenols in plants has been discussed by a number of authors, including GALSTON (1969), GROSS (1975), KEFELI and KUTACEK (1977), MCCLURE (1975) and STENLID (1970). Only a brief summary of these physiological interactions need be given here.

Do phenolics have a role as endogenous hormones in the plant kingdom? From most considerations, the answer must be no for the common phenolics, and particularly for the flavonoids, since they do not have the requisite properties expected of hormonal material. Nevertheless, it is possible that individual phenols actually function in this way. A case has been made that lunularic acid is a dormin-like hormone in liverworts, replacing the more widespread abscisic acid which is absent from these plants (PRYCE, 1972b). Growth experiments with *Lunularia cruciata* support this hormonal role. The structural resemblance between lunularic and abscisic acids has already been pointed out. Whether other related stilbene-like structures such as the batatasins in *Dioscorea* have a role as dormancy hormones in higher plants is less certain, but further experiments may establish these dibenzyl derivatives as regulators in their own right.

Even if most phenolics are not hormones themselves, they may affect plant growth by interaction with one or other of the major classes of plant hormone such as the auxins. Much attention has been paid to the effect of phenolic compounds on indoleacetic acid oxidase, a peroxidase-type enzyme which is capable of auxin destruction. It is well established that, in vitro, flavonoids with a catechol B-ring have a sparing effect on IAA by inhibiting oxidase activity and hence theoretically they have a stimulating effect on plant growth. By contrast, flavonoids with a monohydroxy phenol B-ring uniformly augment enzyme activity and thus have a potentially inhibiting effect on growth (STENLID, 1968, 1976a). Related hydroxycinnamic acids have similar effects. How important this is in vivo has never been fully examined and indeed the question of whether IAA oxidase has a regulating role in auxin activity has not been satisfactorily answered. An in vitro effect of phenolics on the pathway of auxin biosynthesis from anthranilic acid and tryptophan has also been demonstrated, but again the in vivo significance of such interactions has yet to be established (KEFELI and KUTACEK, 1977).

Another point of hormone control that phenolics might affect is the biosynthesis of ethylene. Thus, it is known that a *p*-coumaric acid ester is a necessary co-factor for ethylene biosynthesis from methionine in cauliflower florets (MAPSON, 1970). It is a cofactor to a peroxidase-like enzyme on the pathway and it is interesting that caffeic acid can inhibit the enzyme activity. Thus, the balance of *p*-coumaric to caffeic acid at the site of the synthesis could theoretically provide a regulation of ethylene synthesis.

Phenolics may react with other hormones by synergism or inhibition and both situations have been recorded in the case of plant growth stimulated by gibberellic acid. There is evidence that dihydroconiferyl alcohol in lettuce has a synergistic effect on the GA_3-stimulated elongation of hypocotyl (KAMISAKA and SHIBATA, 1977); by contrast substitution of dihydroconiferyl alcohol by any one of several common hydroxycinnamic acids reverses this effect. It may be noted also that

tannins have generally been shown in other plant systems to have an antagonist effect on GA_3 activity (CORCORAN et al., 1972).

In the above examples, phenolics appear to interact specifically with plant hormones to produce effects on growth. It is also clear that phenolics may have indirect effects on physiological processes, through more non-specific effects on intermediary metabolism. For example, many phenolics are capable of inhibiting ATP synthesis in mitochondria, of uncoupling respiration and of inhibiting ion absorption in roots (STENLID, 1970). Flavonoids may also affect the polar transport of auxins (STENLID, 1976b) and protoplasmic streaming in root hairs (POPOVICI and REZNIK, 1976). There are also a variety of enzymic activities which may be inhibited in the presence of compounds such as quercetin (VAN SUMERE et al., 1975). How significant these effects are in the normal growth pattern of the plant has yet to be determined.

The relatively recent discovery that certain phenolics, particularly caffeic acid esters and flavonoids, occur in plant chloroplasts (see e.g., SAUNDERS and MCCLURE, 1976) in small amounts raises the question of a further possible function in relationship to photosynthesis or to the effect of light on plant processes. Certainly, it is conceivable that phenolic constituents, because of their intense UV absorption, can provide protection from damaging UV radiation in the atmosphere and may be valuable in absorbing this radiation which might otherwise interfere with the more vital processes in the chloroplast. Their occurrence in the chloroplast may equally well be accidental and further studies are needed to see whether they are in fact of importance in the overall metabolism of these organelles.

6.2 Ecological Significance

While it has been difficult to assign a physiological role for the majority of plant phenolics (Sect 6.1), there is increasing evidence that a considerable number of these substances play an ecological role in plants (Table 7). The importance of flavonoid pigments (together with carotenoids) in contributing to flower and fruit colour for purposes of attracting animals to plants for pollination and seed dispersal was recognised by CHARLES DARWIN and by many naturalists before and since his time. The relationship between flavonoid structure and plant colour has recently been reviewed (HARBORNE, 1976b). The contribution of the coloured flavonoids in the cell vacuoles to flower colour is self-evident, but it has taken longer to recognise that the "colourless" flavones and flavonols universally present in flower tissues are essential as co-pigments to the anthocyanins and are also occasionally concerned as "hidden" UV honey guides for attracting bees to flowers.

Other ecological roles for phenolics (Table 7) are based on more recent observations in nature. A series of experiments in both field and laboratory have indicated during the last decade a role for a number of phenolic derivatives as allelopathic agents. These are chemicals excreted by the plant, which may be autotoxic or affect the growth of other plants in the environment (RICE, 1974). It has also been found that flavonoids, and especially tannins, have a role as feeding deterrents, protecting plants from overgrazing by many animal species (SWAIN, 1977; HARBORNE, 1977a).

Table 7. Ecological importance of phenolics in plants

Role	Phenolic class	Examples and plant source[a]
Flower pigments	Anthocyanins	Cyanidin 3,5-diglucoside in *Rosa*
	Chalcones	Coreopsin in *Coreopsis tinctoria*
	Aurones	Aureusin in *Antirrhinum majus*
	Yellow Flavonols	Gossypetin 7-glucoside in *Gossypium*
	Flavones	Apigenin 7-glucoside in *Bellis perennis*
Fruit pigments	Anthocyanins	Petunidin glycoside in *Atropa belladonna*
	Isoflavones	Osajin in *Maclura pomifera*
	Chalcones	Okanin in *Kyllingia brevifolia*
Allelopathic Agents	Quinones	Juglone in *Juglans regia*
	Phenols	Hydroquinone in *Arctostaphylos*
	Phenolic acids	Salicylic acid in *Quercus falcata*
	Hydroxycinnamic acids	Ferulic acid in *Adenostoma*
Feeding deterrents	Quinones	Juglone in *Carya ovata*
	Tannins	Gallotannins in *Quercus robur*
	Flavonols	Quercetin glycosides in *Gossypium*
Anti-fungal agents	Isoflavones	Luteone in *Lupinus*
	Phenolic acids	Protocatechuic acid in *Allium*
	Dihydrochalcones	Phloridzin in *Malus pumila*
Phytoalexins	Stilbenes	Resveratrol in *Arachis hypogaea*
	Phenanthrenes	Orchinol in *Orchis militaris*
	Isoflavans	Vestitol in *Lotus corniculatus*
	Pterocarpans	Pisatin in *Pisum sativum*
	Phenylpropanoids	Coniferyl alcohol in *Linum usitatissimum*
	Furocoumarins	Psoralen in *Petroselinum crispum*

[a] In most cases, many other examples could be quoted. References are given in the text or may be found in SWAIN (1977) and HARBORNE (1976b, 1977a)

Finally, yet another important function for certain classes of phenol is as antimicrobial agents, in providing resistance to various fungal, bacterial, and viral infections. Phenolics are significant here not only as preformed antifungal compounds (SCHÖNBECK and SCHLÖSSER, 1976) but also as phytoalexins formed post-infectionally. Phenolic phytoalexins of one type or another (Table 7) have been recognised in being formed on fungal infection in at least six different plant families (HARBORNE and INGHAM, 1978). See also the chapter on phytoalexins in Vol. 4 (pp. 636–652) of this Encyclopedia (1976).

The ecological importance of phenolics, together with that of other secondary constituents, has been reviewed at length elsewhere (see e.g., SWAIN, 1977 and references therein). A few selected examples are illustrated in Table 7; the structures of many of the compounds listed here may be found in earlier sections of this chapter. In conclusion, it may be pointed out that the ecological effectiveness of many phenolics may well lie in their ability to modify growth processes in other organisms through hormonal interactions. Thus progress in understanding the ecological functions of phenolics will undoubtedly illuminate at the same time our comprehension of their physiological importance in the plant.

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